

*Full Paper*

## **1-(6-choloroquinoxaline-2-yl) Hydrazine as an Excellent Ionophore for Ppreparation of a Cobalt Selective Electrode and Potentiometric Measuring of Vitamin B<sub>12</sub> in Pharmaceutical Samples**

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**Abstract-** In this study, a potentiometric cobalt selective membrane sensor was prepared based on 1-(6-choloroquinoxaline-2-yl) hydrazine as a new ionophore. At first, the lowest-energy conformer of 1-(6-choloroquinoxaline-2-yl) hydrazine molecule (Ligand) and four local structure minima corresponding to their complexes with various ions were obtained using density functional theory (DFT). The calculated results of the Gibbs free energy of the reaction at B3LYP/6-31G(d) level show that the thermodynamic reactivity of complexation of cobalt ion (Co<sup>2+</sup>) with studied ligand is the best. Finally, a liquid membrane electrode for potentiometric monitoring of ultra-trace amounts of Co<sup>2+</sup> was prepared by using this ligand. The electrode exhibits a Nernstian response for Co<sup>2+</sup> ion over a wide concentration range of 1.0×10<sup>-1</sup> to 3.0×10<sup>-6</sup> mol L<sup>-1</sup>. The limit of detection of the sensor is 1.0×10<sup>-6</sup> mol L<sup>-1</sup>. The sensor has a relatively fast response time (~10 s) and a useful working pH range of 3.0-8.0. Interferences of some cations were also evaluated. It was used as an indicator electrode in potentiometric titration of Co<sup>2+</sup> with EDTA and in indirect determination of vitamin B<sub>12</sub> in pharmaceutical samples. The results indicate that this electrode is sensitive for determination of Co<sup>2+</sup> and vitamin B<sub>12</sub>.

**Keywords-** 2-(6-choloroquinoxaline-2-yl) hydrazine, Cobalt selective electrode, Liquid membrane, Potentiometry, Vitamin B<sub>12</sub>

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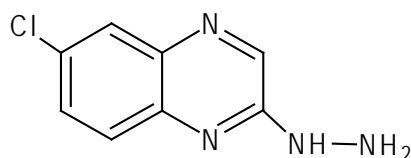
## 1. INTRODUCTION

Vitamin B<sub>12</sub> is a water-soluble vitamin which is essential to growth, cell production, hematopoiesis and myelin synthesis [1]. Vitamin B<sub>12</sub> is the largest and most complex vitamins and its most important sources are liver, meat, egg, fish, dairy products and other animal food sources [2]. This vitamin is used for treating pernicious anemia, cyanide poisoning, trigeminal neuralgia and vitamin B<sub>12</sub> deficiency [3-6]. Vitamin B<sub>12</sub> contains the biochemically rare element cobalt positioned in the center of corrin ring which is synthesized by bacteria and this complex gives the red color to this vital vitamin [7]. Vitamin B<sub>12</sub> has different forms that are similar to each other in the cobalt central ion, corrin ring and a dimethylbenzimidazol group, but has a difference in the sixth site that can be consisted of hydroxyl group (OH), cyano group (CN), methyl group (CH<sub>3</sub>) and 5'-deoxyadenosyl group (C-CO). These forms are called hydroxycobalamin, cyanocobalamin, methylcobalamin and adenosylcobalamin respectively [8]. Different instrumental methods such as atomic absorption spectrometry (AAS) [9], inductive-coupled plasma-mass spectrometry (ICP-MS) [10], Radioimmunoassay (RIA) [11], high-performance liquid chromatography (HPLC) [12], capillary electrophoresis [13], spectrophotometry [14], voltammetry [15] and microbiological methods [16] have been used for vitamin B<sub>12</sub> determination. The most of the mentioned methods are time consuming and elaborate and involve sophisticated and expensive equipments that might not be available in most analytical laboratories. In addition some techniques like spectrophotometry and microbiological methods suffer from poor precision, low specificity [7] and required pre-sample treatment and even required an expert operator for handling the instruments.

On the other hand, potentiometric ion selective electrodes offer several advantages over other methods of analysis. The most significant features of this technique are the speed with which the samples can be analyzed, low cost, simplicity, portability of the device, wide working range, sample non destruction, good precision, online monitoring and high selectivity. The increasing use of ion selective electrodes in the various fields has generated increasing interest in the development new sensors [17-29] to the fast and accurate determination of various species. Some commercial sensors for heavy metals, halides, alkali and alkaline earth metals, etc. are available. However more efforts are required to develop ion selective electrodes for drugs and vitamins, because the quality of dietary supplements and pharmaceutical products is a very significant problem all over the world. Also, determining the content of active ingredient plays a key role in estimating the quality and efficacy of a medicinal product [30]. On the basis of the literature, only one scientific work has been reported on vitamin B<sub>12</sub> determination by ion selective electrodes [31].

Therefore in this work, a potentiometric method to vitamin B<sub>12</sub> determination was reported. At first, the theoretical thermodynamic properties of the reaction complexation of Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup> ions with 1-(6-

chloroquinoxaline-2-yl) hydrazine molecule in gas and liquid phases are studied with B3LYP/6-31G(d) computational level for all atoms except  $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions where B3LYP/LANL2DZ computational level are used. Finally, a liquid membrane ion selective electrode based on 1-(6-chloroquinoxaline-2-yl) hydrazine (Fig. 1) as a new excellent ionophore was prepared and used to measuring of  $\text{Co}^{2+}$  and vitamin  $\text{B}_{12}$ . To the best our knowledge, this is the first application of this ionophore for determination of cobalt and vitamin  $\text{B}_{12}$ .



**Fig. 1.** 1-(6-chloroquinoxaline-2-yl) hydrazine molecule

## 2. EXPERIMENTAL

### 2.1. Apparatus

UV-Visible spectra were recorded by Varian Cary100-Bio UV-Visible spectrophotometer. A corning ion analyzer 250 pH/mV meters was used for potential measurements at  $25.0 \pm 0.1$  °C. Two Ag/AgCl reference electrodes (Azar-Electrode, Iran) were used as the internal and external reference electrode.

### 2.2. Reagents and Materials

Reagent grade dibutyl phthalate (DBP), benzyl acetate (BA), nitrobenzene (NB), acetophenone (AP), sodium tetraphenyl borate (NaTPB), oleic acid (OA), high relative molecular weight polyvinyl chloride (PVC) and tetrahydrofuran (THF) were purchased from Merck and used as received. 1-(6-chloroquinoxaline-2-yl) hydrazine was synthesized and purified as described elsewhere [32]. Nitrate salts of the cations used (from Merck and Aldrich) were all of the highest purity available, and used without any further purification except for vacuum drying over  $\text{P}_2\text{O}_5$ . Doubly distilled deionized water was used throughout.

### 2.3. Investigating the Selectivity of the Ionophore

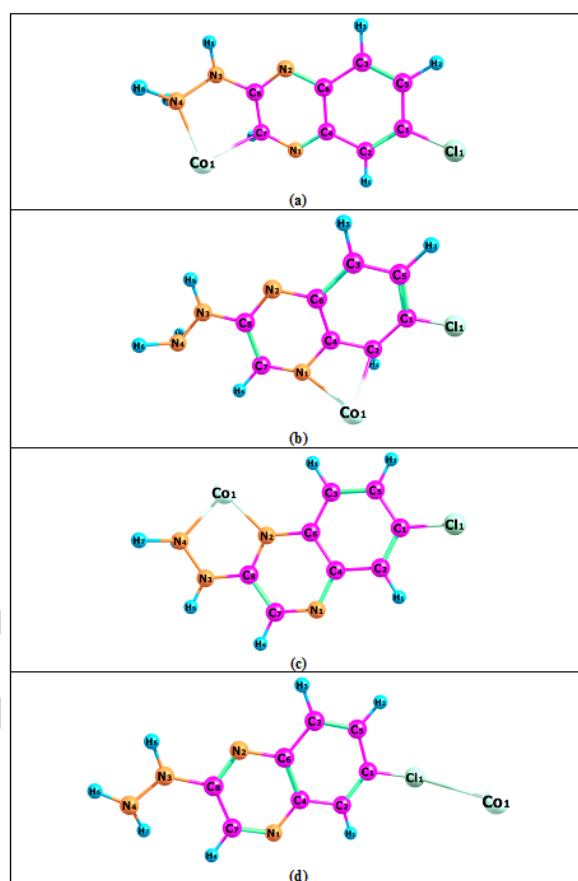
#### 2.3.1. UV-Visible spectrophotometry

The interaction of ionophore 1-(6-chloroquinoxaline-2-yl) hydrazine and some metal ions was investigated by UV-Visible spectrophotometry as followed: 300  $\mu\text{l}$  of a solution of  $10^{-3}$  mol  $\text{L}^{-1}$   $\text{Co}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  was added to a

solution of ligand at a concentration of  $5 \times 10^{-5}$  mol L<sup>-1</sup> and variations in the ligand spectrum were measured in the range of 200-800 nm.

### 2.3.2. Methods and Computations

We obtained the equilibrium geometry of 1-(6-chloroquinoxaline-2-yl) hydrazine molecule (Ligand) (Fig. 1) and four local structure minima corresponding to their complexes with Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup> ions (Fig. 2) by density functional theory (DFT) in the gas and liquid phases performed using the exchange-correlation potential that constructed from Becke's three parameter functional for exchange (B3) [33,34] along with the Lee-Yang-Parr parameterization for correlation (LYP) [35] as implemented B3LYP method.



**Fig. 2.** Four local structure minimum complex of metal ions (for example: Co<sup>2+</sup>) with 1-(6-chloroquinoxaline-2-yl) hydrazine ligand

These calculations are performed by 6-31G(d) basis set for all atoms except Ag<sup>+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup> ions where LANL2DZ (Los Alamos National Laboratory of Double Zeta)

basis set are used. The frequencies were computed to assess thermodynamic properties molecules at the same theoretical level and ensure the nature of local minima (lowest frequency in Table 1 and Table 3).

All DFT calculations in the gas and liquid phases were performed using Gaussian 09 software [36]. The liquid phase is simulated by Polarizable Continuum Model (PCM) method using the integral equation formalism variant (IEFPCM). This method creates the solute cavity via a set of overlapping spheres [37].

#### 2.4. Electrode Preparation

The general procedure to prepare the PVC membrane was as followed: different amounts of the ionophore 1-(6-chloroquinoxaline-2-yl) hydrazine along with appropriate amounts of additive (NaTPB or OA) were weighed. Afterward, known amounts of plasticizer and PVC were added to the mixture. The mixture was dissolved in 4 mL of tetrahydrofurane (THF), and the solution was blended well. The resulting mixture was transferred to a glass dish of 2 cm diameter. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A sampler tip (3-5 mm o.d.) was dipped in to the mixture for about 5 s so that a transparent membrane of about 0.3 mm thickness was formed. The sampler tip was then pulled out from the mixture and kept at room temperature for about 24 h. afterward the sampler tip was filled with an internal filling solution ( $1.0 \times 10^{-3}$  mol L<sup>-1</sup> Co(NO<sub>3</sub>)<sub>2</sub>) solution. The electrode was finally conditioned for 24 h by soaking in a  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> Co (NO<sub>3</sub>)<sub>2</sub> solution.

#### 2.5. Emf Measurements

The following cell was assembled for the conduction of the emf (electromotive force) measurements; Ag-AgCl, KCl (satd.) | internal solution,  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> Co(NO<sub>3</sub>)<sub>2</sub> | PVC membrane | sample solution | Ag-AgCl, KCl (satd.)

#### 2.6. Analytical procedure

Vitamin B<sub>12</sub> was determined indirectly by measurement of the cobalt ions, in three pharmaceutical products: Vitamin B<sub>12</sub> ampoule (Darou Paksh, Iran), Ravit-B ampoule (Raha Pharma, Iran) and Vitamin B<sub>12</sub> Sublingual tablet (Webber Naturals, Canada).

**Table 1.** Total energy and lowest frequency of complexes, and distances of metal ions to nitrogen atoms of ligand in 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> local structure minimum complexes and distances of metal ions to chlorine atom of the ligand in 4<sup>th</sup> local structure minimum complex in gas phase by B3LYP method

Complexes	Local structure	Total energy (a.u.)	Lowest frequency (cm <sup>-1</sup> )	M-N1(Å)	M-N2(Å)	M-N3(Å)	M-N4(Å)	M-Cl(Å)
Cu <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2627.90192	57.8	4.658	1.880	2.661	1.974	
	2 <sup>nd</sup>	-2627.87265	50.8	1.948	4.197	5.372	6.005	
	3 <sup>rd</sup>	-2627.86451	49.2	2.904	3.805	2.678	1.968	
	4 <sup>th</sup>	-2627.84028	25.3					2.081
Zn <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2766.73618	59.6	4.652	1.904	2.712	2.039	
	2 <sup>nd</sup>	-2766.69843	57.0	3.004	3.797	2.756	2.117	
	3 <sup>rd</sup>	-2766.69766	54.8	2.030	4.512	5.575	5.969	
	4 <sup>th</sup>	-2766.67465	26.1					2.751
Ni <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2495.72668	55.9	4.499	1.790	2.687	1.908	
	2 <sup>nd</sup>	-2495.69018	54.3	2.652	3.826	2.708	1.898	
	3 <sup>rd</sup>	-2495.68263	47.1	1.844	4.255	5.320	5.729	
Co <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2370.24695	56.4	4.680	1.896	2.756	2.060	
	2 <sup>nd</sup>	-2370.20966	52.7	1.951	4.260	5.383	5.853	
	3 <sup>rd</sup>	-2370.19710	55.3	2.948	3.835	2.791	2.065	
	4 <sup>th</sup>	-2370.15818	36.2					2.278
Fe <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2251.21738	49.0	4.725	1.938	2.857	2.147	
	2 <sup>nd</sup>	-2251.18253	53.6	2.002	4.371	5.471	5.900	
	3 <sup>rd</sup>	-2251.17146	53.7	2.984	3.828	2.821	2.128	
	4 <sup>th</sup>	-2251.15243	37.9					2.273
Hg <sup>2+</sup> -Ligand	1 <sup>st</sup>	-1030.34251	15.8	6.756	4.753	3.949	3.012	
Mn <sup>2+</sup> -Ligand	1 <sup>st</sup>	-2138.56128	57.5	4.772	1.989	2.884	2.197	
	2 <sup>nd</sup>	-2138.52400	53.7	3.045	3.889	2.913	2.220	
	3 <sup>rd</sup>	-2138.51711	54.3	2.060	4.478	5.551	5.955	
	4 <sup>th</sup>	-2138.51212	21.8					2.895
Cd <sup>2+</sup> -Ligand	1 <sup>st</sup>	-1035.66548	50.0	5.017	2.254	3.096	2.406	
	2 <sup>nd</sup>	-1035.64407	14.6	5.559	5.348	3.735	2.812	
	3 <sup>rd</sup>	-1035.64350	41.5	2.460	5.261	5.734	5.833	
Mg <sup>2+</sup> -Ligand	1 <sup>st</sup>	-1187.75717	58.6	4.759	1.989	2.809	2.137	
	2 <sup>nd</sup>	-1187.71887	57.6	2.056	4.535	5.580	5.963	
	3 <sup>rd</sup>	-1187.70751	56.3	3.135	3.831	2.855	2.182	
	4 <sup>th</sup>	-1187.68981	39.3					2.758
Pb <sup>2+</sup> -Ligand	1 <sup>st</sup>	-991.12919	28.0	5.046	2.241	3.199	3.199	
	2 <sup>nd</sup>	-991.09503	50.7	2.320	4.690	5.729	5.729	
	3 <sup>rd</sup>	-991.07770	42.5	3.186	4.225	3.276	3.276	
	4 <sup>th</sup>	-991.07487	30.4					2.684
Ca <sup>2+</sup> -Ligand	1 <sup>st</sup>	-1665.28533	47.1	5.170	2.368	3.238	3.238	
	2 <sup>nd</sup>	-1665.25754	54.3	2.397	4.859	5.852	5.852	
	3 <sup>rd</sup>	-1665.25149	40.9	3.933	3.715	2.413	2.413	
	4 <sup>th</sup>	-1665.21837	28.8					2.855
Ag <sup>+</sup> -Ligand	1 <sup>st</sup>	-1133.80025	54.4	5.060	2.262	3.117	3.117	
	2 <sup>nd</sup>	-1133.78413	37.2	2.198	4.995	5.516	5.516	
	3 <sup>rd</sup>	-1133.77161	37.6	3.304	3.916	3.128	3.128	
	4 <sup>th</sup>	-1133.75468	27.5					2.582

An accurately weighed sample was transferred into a 100-mL beaker, and then 5 mL of concentrated sulfuric acid was added, and the mixture was heated until the solution got carbonized and white fumes of  $\text{SO}_3$  were evolved. Afterward if the hot digest was not colorless, above procedure would be repeated again. After the digest got cooled, it was dissolved in 25 mL of deionized water. Then the pH of the solution was adjusted to neutral with concentrated sodium hydroxide solution [9].

### 3. RESULTS AND DISCUSSION

#### 3.1. Theoretical results

The calculations were done for each of the reactants (Ligands and Metal Ions) and products (Complexes) in the reaction:



Generally four local structure minimum complexes that ligand bonded to ion metals are obtained (Fig. 2).

In the first stage, the reaction in the gas phase has been investigated, while in the second step the reaction has been studied in the solution phase. In liquid phase, metal ions are considered in water solvent, while ligands and Complexes are studied in nitrobenzene solvent. Distances of metal ions to nitrogen atoms of ligand in first, second and third local structure minimum complexes and distance of metal ions to chlorine atom of ligand in fourth local structure minimum complex (as shown for  $\text{Co}^{2+}$  in Fig. 2) in gas phase are tabulated in Table 1. Also the thermo chemistry output in the first local structure minimum complex (with the lowest total energy) in gas phase is summarized in Table 2.

In this table,  $\Delta\varepsilon_0$  is variation of total electronic energy;  $\Delta H_{\text{th}}$ ,  $\Delta S_{\text{th}}$  and  $\Delta G_{\text{th}}$  are variation of thermal corrections to the enthalpy, entropy and Gibbs free energy, respectively;  $\Delta G_0$  is Gibbs free energy and  $K_f$  is equilibrium constant of above reaction at standard state (298 K and 1 atm). For calculating the thermal correction of Gibbs free energy, the entropy term was added to thermal correction of enthalpy ( $\Delta G_{\text{th}} = \Delta H_{\text{th}} - T\Delta S_{\text{th}}$ ). Also Gibbs free energy is equal with summation of the variation of the total electronic energy in the reaction and thermal correction of the Gibbs free energy ( $\Delta G_0 = \Delta\varepsilon_0 + \Delta H_{\text{th}} - T\Delta S_{\text{th}}$ ) and equilibrium constant is obtained as:  $K_f = \exp(-\Delta G_0/RT)$ .

The variations of thermal correction to entropy complexes formation from all of ion metals with ligand are negative, so the aforementioned reaction the entropy of complexation due to aggregation in complex is inappropriate, but the enthalpy of the reaction at standard state ( $\Delta H_0 = \Delta\varepsilon_0 + \Delta H_{\text{th}}$ ) is appropriate to obtain a complex from ligand and ion metal, because the enthalpy of the reaction complexes formation from all of ion metals with ligand at standard state are negative. Therefore the results of the Gibbs free energy and equilibrium constant of the reaction show that the thermodynamic reactivity of complexation of ion

metals with studied ligand (with the lowest total energy) are:  $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Hg}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+} > \text{Ca}^{2+} > \text{Ag}^+$  respectively.

**Table 2.** Calculated thermo chemistry values for the reaction: Ligand+Metal ion  $\rightarrow$  Complex in 1<sup>st</sup> local structure minimum complex (with the lowest total energy) in gas phase by B3LYP method

Complexes	$\Delta\epsilon_0$ (kJ/mol)	$\Delta H_{th}$ (kJ/mol)	$\Delta S_{th}$ (J/mol K)	$\Delta G_{th}$ (kJ/mol)	$\Delta G_0$ (kJ/mol)	$K_f$
$\text{Cu}^{2+}$ -Ligand	-1181.871	492.382	-127.666	40.850	-1141.020	8.117E+199
$\text{Zn}^{2+}$ -Ligand	-1025.101	4.255	-129.523	34.895	-990.206	3.064E+173
$\text{Ni}^{2+}$ -Ligand	-1007.362	493.192	-135.470	43.554	-963.807	7.264E+168
$\text{Co}^{2+}$ -Ligand	-995.788	-53.166	-121.750	38.899	-956.889	4.457E+167
$\text{Fe}^{2+}$ -Ligand	-881.357	490.633	-119.604	37.631	-843.725	6.645E+147
$\text{Hg}^{2+}$ -Ligand	-814.839	492.759	-91.077	30.120	-784.719	3.052E+137
$\text{Mn}^{2+}$ -Ligand	-785.747	4.305	-124.259	33.374	-752.373	6.567E+131
$\text{Mg}^{2+}$ -Ligand	-764.401	491.820	-121.821	38.844	-725.557	1.315E+127
$\text{Cd}^{2+}$ -Ligand	-711.307	489.491	-117.851	36.576	-674.731	1.638E+118
$\text{Pb}^{2+}$ -Ligand	-622.378	489.772	-112.068	34.982	-587.396	8.184E+102
$\text{Ca}^{2+}$ -Ligand	-472.034	491.235	-113.742	36.164	-435.870	2.320E+76
$\text{Ag}^+$ -Ligand	-230.434	493.086	-115.093	37.432	-193.003	6.523E+33

The order of the arrangement of complexes in Fig. 2 in terms of total energy (Table 1) is: (a) first, (b) second, (c) third and (d) fourth. Also based on the results of Table 1, it is clear that for the mercury complex only the first stable structure was obtained and the fourth structure was not optimized in the cadmium complex.

**Table 3.** The average calculated thermo chemistry values by Boltzmann population analysis for the reaction: Ligand + Metal ion  $\rightarrow$  Complex in all of local structure minimum complexes in gas phase by B3LYP method

Complexes	$\Delta\epsilon_0$ (kJ/mol)	$\Delta H_{th}$ (kJ/mol)	$\Delta S_{th}$ (J/mol K)	$\Delta G_{th}$ (kJ/mol)	$\Delta G_0$ (kJ/mol)	$K_f$
$\text{Cu}^{2+}$ -Ligand	-1098.994	486.280	-115.670	34.385	-1064.394	3.049E+186
$\text{Zn}^{2+}$ -Ligand	-936.008	3.143	-119.235	24.473	-907.886	1.158E+159
$\text{Ni}^{2+}$ -Ligand	-937.903	489.850	-136.728	42.371	-895.496	7.812E+156
$\text{Co}^{2+}$ -Ligand	-883.107	-55.779	-115.734	34.464	-848.353	4.298E+148
$\text{Fe}^{2+}$ -Ligand	-787.265	485.884	-114.400	33.842	-753.249	9.351E+131
$\text{Hg}^{2+}$ -Ligand	-814.839	492.759	-91.077	30.120	-784.719	3.052E+137
$\text{Mn}^{2+}$ -Ligand	-701.059	2.439	-113.355	28.200	-672.732	7.313E+117
$\text{Cd}^{2+}$ -Ligand	-673.634	489.529	-102.562	32.016	-641.548	2.515E+112
$\text{Mg}^{2+}$ -Ligand	-664.159	487.984	-112.244	34.163	-629.779	2.181E+110
$\text{Pb}^{2+}$ -Ligand	-531.815	487.245	-111.540	33.641	-498.119	1.868E+87
$\text{Ca}^{2+}$ -Ligand	-389.195	488.065	-107.678	32.848	-356.136	2.488E+62
$\text{Ag}^+$ -Ligand	-171.924	491.136	-104.178	33.250	-138.576	1.900E+24



The average calculated thermo chemistry values by Boltzmann population analysis for the reaction: Ligand + Metal ion  $\rightarrow$  Complex, in all of four local structure minimum complexes in gas phase (Table 3) show the sequence thermodynamic reactivity of complexation of ion metals with the studied ligand is as before, with the only differences that the magnesium and cadmium reactivity sequence is reversed and Gibbs free energy are shifted to lower values.

Because the Boltzmann population analysis in all of four local structure minimum complexes in gas phase only displaced energy, but did not affect the reactivity order, only the most stable complex (the first one) in the liquid phase was investigated. Distances of metal ions to nitrogen atoms of ligand in first local structure minimum complex with the lowest total energy (as shown for  $\text{Co}^{2+}$  in Fig. 2) in liquid phase are tabulated in Table 4.

**Table 4.** The lowest frequency of complexes, and distances of metal ions to nitrogen atoms of ligand in 1<sup>st</sup> local structure minimum complex (with the lowest total energy) in liquid phase by B3LYP method

Complexes	Lowest frequency (cm <sup>-1</sup> )	M-N1 (Å)	M-N2 (Å)	M-N3 (Å)	M-N4 (Å)
$\text{Co}^{2+}$ -Ligand	60.6	4.840	2.044	2.907	2.146
$\text{Ni}^{2+}$ -Ligand	65.3	4.592	1.815	2.687	1.861
$\text{Cu}^{2+}$ -Ligand	63.2	4.702	1.920	2.755	1.984
$\text{Pb}^{2+}$ -Ligand	27.5	5.145	2.330	3.210	2.426
$\text{Mg}^{2+}$ -Ligand	49.6	4.891	2.092	2.898	2.145
$\text{Zn}^{2+}$ -Ligand	60.2	4.796	2.002	2.835	2.113
$\text{Fe}^{2+}$ -Ligand	81.8	4.885	2.088	2.951	2.186
$\text{Mn}^{2+}$ -Ligand	69.9	5.012	2.207	3.044	2.268
$\text{Ag}^+$ -Ligand	51.6	5.156	2.341	3.181	2.449
$\text{Ca}^{2+}$ -Ligand	80.9	5.465	2.640	3.520	3.520
$\text{Cd}^{2+}$ -Ligand	64.0	5.219	2.409	3.221	2.461
$\text{Hg}^{2+}$ -Ligand	93.8	5.367	2.550	3.354	2.590

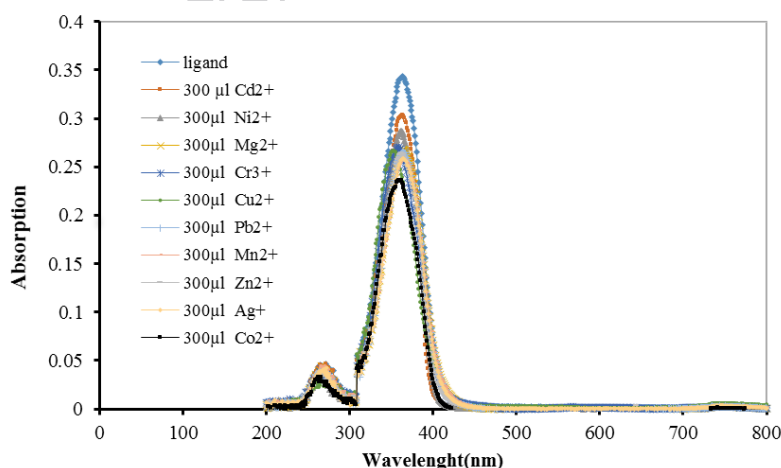
The Calculated thermo chemistry values for the reaction: Ligand+Metal ion $\rightarrow$ Complex in first local structure minimum complex (with the lowest total energy) in liquid phase is summarized in Table 5. The results of the Gibbs free energy and equilibrium constant of the reaction show that the thermodynamic reactivity of complexation of ion metals with studied ligand in liquid phase (metal ions in water solvent, while ligands and Complexes in nitrobenzene solvent) are:  $\text{Co}^{2+}>\text{Ni}^{2+}>\text{Cu}^{2+}>\text{Pb}^{2+}>\text{Mg}^{2+}>\text{Zn}^{2+}>\text{Fe}^{2+}>\text{Mn}^{2+}>\text{Ag}^+>\text{Ca}^{2+}>\text{Cd}^{2+}>\text{Hg}^{2+}$  respectively. Also, silver, calcium, cadmium and mercury ions, tend to form complexes with studied ligand do not show.

**Table 5.** Calculated thermo chemistry values for the reaction: Ligand+Metal ion→Complex in 1<sup>st</sup> local structure minimum complex (with the lowest total energy) in liquid phase by B3LYP method

Complexes	$\Delta\epsilon_0$ (kJ/mol)	$\Delta H_{th}$ (kJ/mol)	$\Delta S_{th}$ (J/mol K)	$\Delta G_{th}$ (kJ/mol)	$\Delta G_0$ (kJ/mol)	$K_f$
Co <sup>2+</sup> -Ligand	-582.870	-50.685	-135.729	45.547	-537.323	1.381E+94
Ni <sup>2+</sup> -Ligand	-243.884	-47.212	-153.616	54.356	-189.529	1.606E+33
Cu <sup>2+</sup> -Ligand	-223.119	-49.488	-137.670	47.322	-175.797	6.310E+30
Pb <sup>2+</sup> -Ligand	-193.027	-49.590	-124.005	43.147	-149.880	1.816E+26
Mg <sup>2+</sup> -Ligand	-192.421	-50.076	-124.955	42.945	-149.476	1.543E+26
Zn <sup>2+</sup> -Ligand	-170.353	-49.824	-136.265	46.569	-123.784	4.867E+21
Fe <sup>2+</sup> -Ligand	-120.390	-54.298	-142.382	43.919	-76.471	2.500E+13
Mn <sup>2+</sup> -Ligand	-75.586	-50.929	-134.725	45.004	-30.582	2.280E+05
Ag <sup>+</sup> -Ligand	-24.750	-54.715	-128.227	39.280	14.531	2.846E-03
Ca <sup>2+</sup> -Ligand	-17.383	-51.998	-125.558	41.202	23.819	6.711E-05
Cd <sup>2+</sup> -Ligand	15.165	-49.556	-126.394	43.893	59.058	4.497E-11
Hg <sup>2+</sup> -Ligand	54.428	-49.906	-137.808	46.947	101.374	1.734E-18

### 3.2. Investigation of the Interaction of Ionophore and Some Metals by UV-Visible Spectrophotometry

With UV-Visible spectra, as illustrated in Fig. 3, it was possible to distinguish the interaction between the ionophore and different ions. Fig. 3 shows the Change in 1-(6-chloroquinoxaline-2-yl) hydrazine absorption in the presence of various ions at a wavelength range of 200-800 nm.

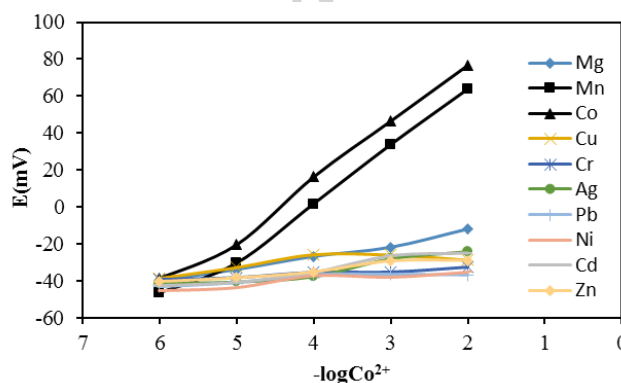


**Fig. 3.**  $5 \times 10^{-5}$  mol L<sup>-1</sup> solution of the ligand UV-Visible spectrum variations by adding 300 µl of a  $10^{-3}$  mol L<sup>-1</sup> solution of different ions

As can be seen, the greatest change in the spectrum achieved after the addition of cobalt ion. This changes show that there is greatest interaction between the ionophore and cobalt ion and can be due to the formation of a ligand-cobalt complex in solution.

### 3.3. The Use of Ionophore in the Membrane Preparation for Various Ions

Despite the fact that, ion carrier plays the most important role in the performance of ion selective electrodes, but other factors such as surface membrane properties, type and amount of ionic additive, type of polymer matrix and plasticizer and their ratio to each other, etc. have a significant effect on the sensor response. For this reason, after investigating the selectivity of ionophore with UV-Visible spectrometry, the proposed sensor's response was directly investigated for a number of different metals. For this purpose, a membrane with a composition of 8% Ionophore, 2% NaTPB, 60% DBP and 30% PVC was prepared and its response to  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$  and  $\text{Cd}^{2+}$  ions were examined. As can be seen clearly in Fig. 4, the best response of sensor is to  $\text{Co}^{2+}$ . A linear relationship with a nernstian slope is seen between the potential and the activity of cobalt ions in a relatively wide concentration range, while the obtained potential responses for other cations are much lower than that predicted by the Nernst equation. This phenomenon indicates that the ionophore has a selective interaction with cobalt ion.



**Fig. 4.** Potential response of various ion selective electrodes based on 2-(6-chloroquinoxaline-2-yl) hydrazine

### 3.4. Membrane Composition Effect on the Potential Response of the Sensor

Because the degree of sensitivity and selectivity for a certain ionophore is greatly related to the membrane ingredients, the influence of membrane composition on the potential responses of the  $\text{Co}^{2+}$  sensor was investigated [38,39]. In this study, different membrane compositions, as shown in table 6, were tested. As can be seen, the membrane with the composition of 30% PVC, 8% Ionophore, 2% NaTPB and 60% DBP which shows the best

Nernstian slope ( $29.4 \pm 0.4$  mV/Decade) was the optimum one in the development of this sensor.

The  $\text{Co}^{2+}$  ion extraction into the liquid membrane is a result of the high concentration of the ligand in the membrane. From table 6, it was obvious that in the absence of ionophore (no. 1,2), the response of the recommended electrode was low (slope of  $10.8 \pm 0.5$  and  $12.1 \pm 0.4$ ) which show significant effect of the ionophore. The sensitivity of the electrode response increases with increasing ionophore content until a value of 8% is reached. It shows the affinity of the ionophore to  $\text{Co}^{2+}$  ion. Further addition of ionophore will, however, result in diminished response of the sensor (no. 6, 7), most probably due to inhomogeneity and possible saturation of the membrane [17].

**Table 6.** The optimization of the membrane ingredients

Membrane No.	Ionophore (%wt)	Ionic additive (%wt)	Plasticizer (%wt)	PVC (%wt)	Slope (mV.Decade <sup>-1</sup> )
1	0	0	(DBP)60	40	10.8( $\pm 0.5$ )
2	0	(NaTPB)2	(DBP)60	38	12.1( $\pm 0.4$ )
3	8	0	(DBP)60	32	23.1( $\pm 0.4$ )
4	5	(NaTPB)2	(DBP)63	30	15.7( $\pm 0.3$ )
<b>5</b>	<b>8</b>	<b>(NaTPB)2</b>	<b>(DBP)60</b>	<b>30</b>	<b>29.4(<math>\pm 0.4</math>)</b>
6	10	(NaTPB)2	(DBP)58	30	22.0( $\pm 0.3$ )
7	12	(NaTPB)2	(DBP)58	28	24.4( $\pm 0.4$ )
8	8	(NaTPB)2	(BA)60	30	19.3( $\pm 0.3$ )
9	10	(NaTPB)2	(BA)60	28	22.5( $\pm 0.4$ )
10	8	(NaTPB)2	(NB)60	30	20.5( $\pm 0.5$ )
11	10	(NaTPB)2	(NB)60	28	22.6( $\pm 0.3$ )
12	8	(NaTPB)2	(AP)60	30	21.5( $\pm 0.5$ )
13	10	(NaTPB)2	(AP)60	28	22.9( $\pm 0.4$ )
14	8	(OA)2	(DBP)60	30	24.2( $\pm 0.3$ )
15	8	(OA)3	(DBP)60	29	24.7( $\pm 0.6$ )
16	8	(OA)5	(DBP)59	28	25.9( $\pm 0.3$ )

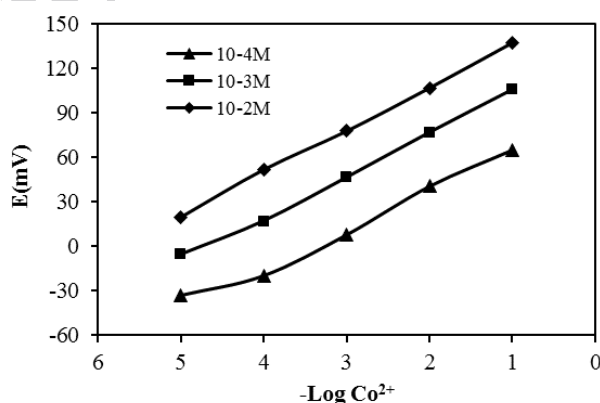
The second factor which helps the extraction of the  $\text{Co}^{2+}$  ions is plasticizer. After the evaluation of four solvent mediators (DBP, NB, BA, AP), it was observed that the DBP displays the better sensitivity than other plasticizers. This indicated that the solvent medium of DBP was probably providing the best complexation environment between  $\text{Co}^{2+}$  and ionophore. It should be noted that the nature of plasticizer influences both the dielectric constant of the membrane and the mobility of ionophore and its complex. NB, BA and AP presented higher dielectric constant values than DBP, leading to the extraction of the polar interfering ions, which could have negative effects on the selectivity behavior of the sensor. This may seem to lead to the poorer extraction of  $\text{Co}^{2+}$  ions, which have a high charge

density, by the average polar solvent. But this seemed to be compensated by the selective complexation of 1-(6-chloroquinoxaline-2-yl) hydrazine with the  $\text{Co}^{2+}$  ions.

The presence of lipophilic anions in a cation-selective membrane was also considered. Actually, the presence of such anions in a cation-selective membrane, which is based on a neutral carrier, decreases the Ohmic resistance and improves the response behavior and selectivity. Furthermore, when the extraction capability is poor, it increases the membrane electrode sensitivity [40]. In this regard, the effect of two ionic additives (OA and NaTPB) was investigated. As can be seen in table 1, NaTPB has a better effect on the sensor response than oleic acid. The presence of 2% NaTPB as proper additive will improve the sensitivity of the  $\text{Co}^{2+}$  sensor significantly and led to the slope increase of the potential sensor response from the sub-Nernstian value of  $23.1 \pm 0.4 \text{ mV}$  per decade (no. 3) to the Nernstian value of  $29.4 \pm 0.4 \text{ mV}$  per decade (no. 5). Actually, it helps to the ion-exchange of the analyte from the aqueous solution to organic layer of the membrane. It's clearly obvious that the sensitivity of the PVC membrane based on the ionophore in the presence of 2%, 3% and 5% OA has dropped significantly (no. 14, 15 and 16).

### 3.5. Internal Solution

The concentration of the internal solution ( $\text{Co}(\text{NO}_3)_2$ ) of the electrode was changed from  $1.0 \times 10^{-2} \text{ mol L}^{-1}$  to  $1.0 \times 10^{-4} \text{ mol L}^{-1}$  and the potential response of the  $\text{Co}^{2+}$  selective electrode was obtained (Fig. 5). It was found that the variation of concentration of the internal solution does not cause any significant differences in potential response, except for an expected change in the intercept of the resulting Nernstian plot. A  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  concentration  $\text{Co}(\text{NO}_3)_2$  solution is quite appropriate for smooth functioning of the electrode system.



**Fig. 5.** The effect of internal reference solution concentration on the electrode response

### 3.6. pH Effect on the Electrode Response

The influence of the pH of the test solution on the potential response of  $\text{Co}^{2+}$  sensor investigated at  $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Co}^{2+}$  ion concentration, in the pH value of 1.0 up to 10.0 (concentrated NaOH or  $\text{HNO}_3$  solutions were employed for the pH adjustment). In agreement with the resulting data (Fig. 6), the potential remained constant despite the pH change in the range of 3.0 to 8.0, indicating the applicability of this electrode in this specific pH range. On the contrary, relatively noteworthy fluctuations in the potential vs. pH behavior took place below and above the formerly stated pH limits. In detail, the fluctuations above the pH value of 8.0 might be justified by the formation of the  $\text{Co}^{2+}$  ion hydroxy complexes in the solution. On the other hand, the fluctuations below the pH value of 3.0 were attributed to the partial protonation of the employed ionophore. If  $\text{H}^+$  concentration is much more than  $\text{Co}^{2+}$ , then  $\text{H}^+$  ions can compete with  $\text{Co}^{2+}$  ions for making complex with ionophore. In pH between 3-8,  $\text{H}^+$  concentration is not enough for competing with  $\text{Co}^{2+}$  ions (because ionophore has more trend to  $\text{Co}^{2+}$  ions in compare to  $\text{H}^+$  ions), but in below pH of 3,  $\text{H}^+$  concentration is enough to overcome on  $\text{Co}^{2+}$  and employed ionophore can be protonated.

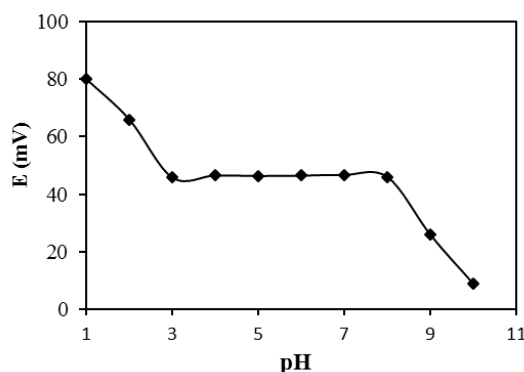
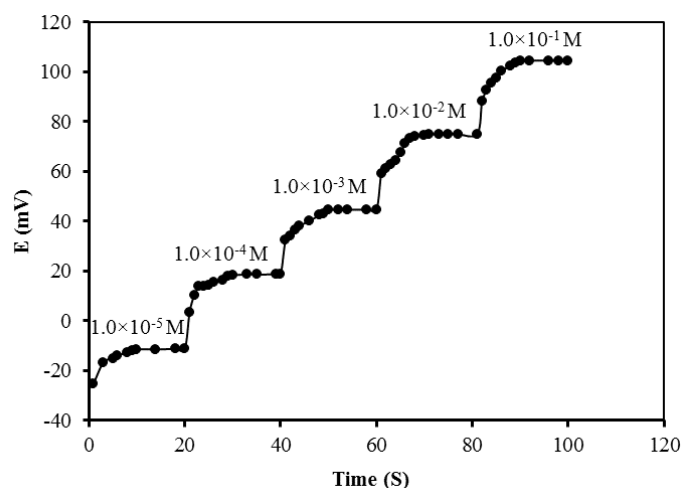


Fig. 6. The effect of the pH on the potential response of the Cobalt sensor

### 3.7. Response Time

The response time of an electrode is evaluated by measuring the average time required to achieve a potential within  $\pm 0.1 \text{ mV}$  of the final steady-state potential, upon successive immersion of a series of interested ions, each having a ten-fold difference in concentration. It is notable that the experimental conditions-like the stirring or flow rate, the ionic concentration and composition of the test solution, the concentration and composition of the solution to which the electrode was exposed before experiment measurements were performed, any previous usages or preconditioning of the electrode, and the testing temperature have an effort on the experimental response time of a sensor [41]. In this work, dynamic response time was obtained by changing the  $\text{Co}^{2+}$  concentration in solution, over a concentration range  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-1} \text{ mol L}^{-1}$ . The actual potential versus time traces is

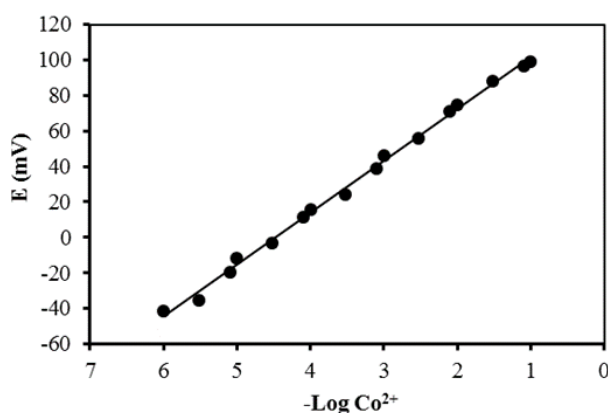
shown in Fig. 7. As can be seen, in whole concentration range the electrode reaches its equilibrium response in a relatively short time ( $\sim 10$ s). This is most probably due to the fast exchange kinetics of complexation-decomplexation of  $\text{Co}^{2+}$  ion with the ionophore at the test solution-membrane interface.



**Fig. 7.** Dynamic response time of Cobalt ion electrode for step changes in concentration of  $\text{Co}^{2+}$  over a concentration range from  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-1}$  mol  $\text{L}^{-1}$

### 3.8. Measuring Range and Detection Limit

The measuring range of an ion-selective electrode includes the linear part of the calibration graph as shown in Fig. 8. The applicable measuring range of the proposed sensor is between  $3 \times 10^{-6}$  and  $1 \times 10^{-1}$  mol  $\text{L}^{-1}$ . In this work the detection limit of the proposed membrane sensor was  $1.0 \times 10^{-6}$  mol  $\text{L}^{-1}$  which was calculated by extrapolating the two segments of the calibration curve.



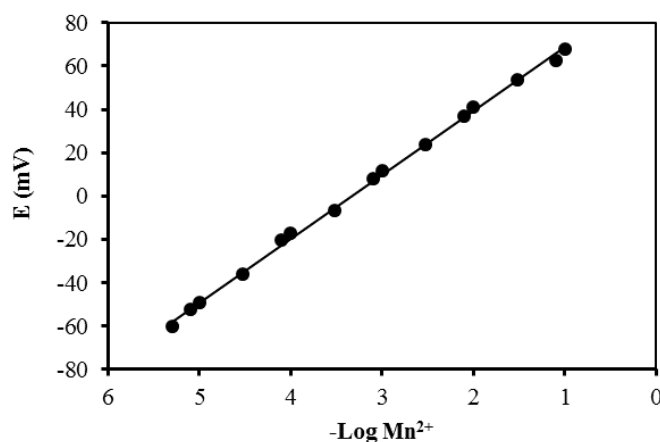
**Fig. 8.** The calibration curve of the  $\text{Co}^{2+}$  membrane sensor

### 3.9. Selectivity

The potentiometric selectivity coefficients of the  $\text{Co}^{2+}$  sensor were evaluated by the matched potential method (MPM) [42]. The calculated selectivity coefficient ( $K_{\text{MPM}}$ ) values are given in Table 7. A value of  $K_{\text{MPM}}=1.0$  indicates equal response to primary and interfering ions. Further, the smaller the value of the selectivity coefficient, the higher is the selectivity of the sensor. It is seen from the table that the selectivity coefficient values are much smaller than 1.0 indicating that they exhibit sufficient selectivity towards  $\text{Co}^{2+}$  over all the interfering ions studied. Of course, it should be noted that the only ion that is competitive with cobalt is  $\text{Mn}^{2+}$ , which can be removed by a masking agent, if it exists in a sample with cobalt.

**Table 7.** The selectivity coefficients of various interfering cations for the  $\text{Co}^{2+}$  membrane sensor

Ion	$K_{\text{MPM}}$
$\text{Cu}^{2+}$	$2.1 \times 10^{-3}$
$\text{Cr}^{3+}$	$1.8 \times 10^{-3}$
$\text{Cd}^{2+}$	$1.3 \times 10^{-3}$
$\text{Mg}^{2+}$	$2.6 \times 10^{-3}$
$\text{Ag}^+$	$2.4 \times 10^{-3}$
$\text{Fe}^{2+}$	$3.2 \times 10^{-3}$
$\text{Pb}^{2+}$	$7.7 \times 10^{-3}$
$\text{Ni}^{2+}$	$3.3 \times 10^{-3}$
$\text{Mn}^{2+}$	$1.1 \times 10^{-1}$
$\text{Zn}^{2+}$	$3.0 \times 10^{-3}$



**Fig. 9.** The calibration curve of the  $\text{Mn}^{2+}$  membrane sensor



### 3.10. Preparation a membrane for determination of Mn<sup>2+</sup>

Due to the great interference that was observed from manganese ions in Co<sup>2+</sup> membrane sensor, it can be concluded that the ionophore also has a good interaction with Mn<sup>2+</sup> and the membrane sensor could be applied for manganese determination. For checking this issue, a membrane with the same composition was prepared and it was conditioned in 10<sup>-3</sup> mol L<sup>-1</sup> Mn<sup>2+</sup> solution for 24 h, and the measuring range and selectivity for Mn<sup>2+</sup> membrane sensor was also evaluated. As can be seen in Fig. 9, the applicable measuring range of the proposed Mn<sup>2+</sup> sensor is between 5×10<sup>-6</sup> and 1×10<sup>-1</sup> mol L<sup>-1</sup>. And the limit of detection was 3.0×10<sup>-6</sup> mol L<sup>-1</sup> which was calculated by extrapolating the two segments of the calibration curve.

The potentiometric selectivity coefficients of the Mn<sup>2+</sup> sensor were evaluated by the matched potential method (MPM) [42]. The calculated selectivity coefficient (K<sub>MPM</sub>) values are given in Table 8. As can be observed the selectivity coefficient values are a little larger than the Co<sup>2+</sup> membrane sensor which can indicate that the ionophore has more affinity to Co<sup>2+</sup> ions than Mn<sup>2+</sup>. However except the cobalt ions which has a significant interference, other ions exhibit sufficient selectivity towards Mn<sup>2+</sup> because their selectivity coefficient values are much smaller than 1.0, which indicates that the sensor can also determine the amount of manganese in a wide concentration range in the absence of Co<sup>2+</sup>.

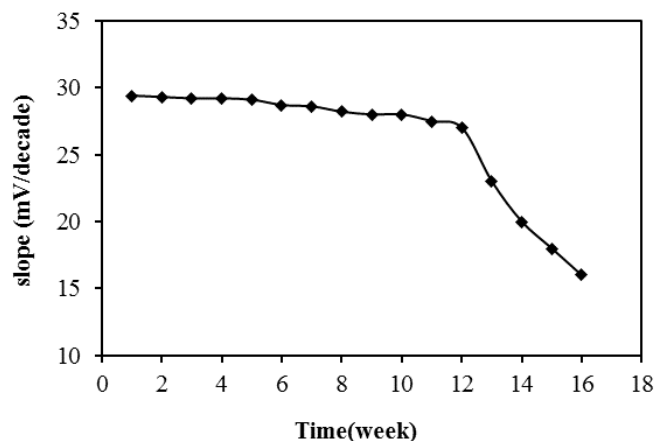
**Table 8.** The selectivity coefficients of various interfering cations for the Mn<sup>2+</sup> membrane sensor

Ion	K <sub>MPM</sub>
Cu <sup>2+</sup>	5.2×10 <sup>-3</sup>
Cr <sup>3+</sup>	5.9×10 <sup>-3</sup>
Cd <sup>2+</sup>	1.3×10 <sup>-3</sup>
Mg <sup>2+</sup>	4.2×10 <sup>-3</sup>
Ag <sup>+</sup>	3.5×10 <sup>-2</sup>
Fe <sup>2+</sup>	3.6×10 <sup>-3</sup>
Pb <sup>2+</sup>	9.5×10 <sup>-3</sup>
Ni <sup>2+</sup>	9.5×10 <sup>-3</sup>
Co <sup>2+</sup>	4.8×10 <sup>-1</sup>
Zn <sup>2+</sup>	3.8×10 <sup>-3</sup>

### 3.11. Lifetime

Lifetime of the membrane electrode was studied by periodically recalibrating and using the electrode to measure its potentiometric response to cobalt ion in the standard solutions in a period of time. After the conditioning step the electrodes were repeatedly calibrated three times a day during a period of four months (the electrode worked one hour per day). As can be seen in Fig. 9. Before 12 weeks no significant change in the performance of the electrode was observed. This shows that the lifetime of the proposed electrode was about 12 weeks.

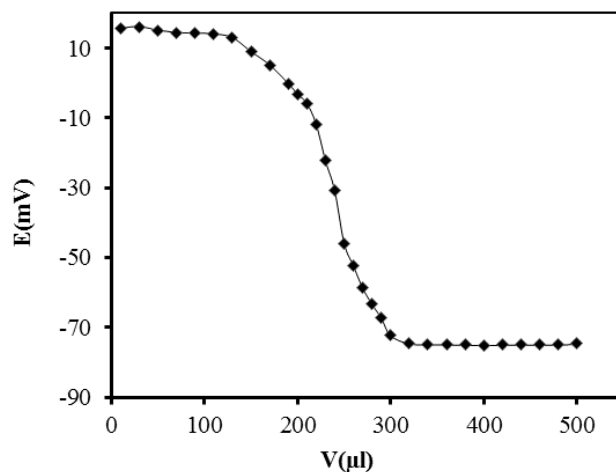
During the 12 weeks, the slope of the cobalt selective electrode decreases from 29.4 to 27.0 mV per decade. It is well established that the loss of plasticizer, carrier, or ionic site from the polymeric film due to leaching into the sample is a primary reason for the limited lifetimes of the sensors.



**Fig. 10** The lifetime of the  $\text{Co}^{2+}$  membrane ion electrode

### 3.12. Analytical Application

Ion-selective electrodes tend to be; low in cost, simple to use, rapid in determination, with low interferences from the matrix and can be applied to small volumes. These characteristics make them an ideal choice for environmental measurements.



**Fig. 11.** Potentiometric titration curve of 25 mL  $1.0 \times 10^{-4}$  mol L<sup>-1</sup>  $\text{Co}^{2+}$  with  $1.0 \times 10^{-2}$  mol L<sup>-1</sup> EDTA, using the proposed sensor as an indicator electrode

### 3.12.1. Potentiometric titration $\text{Co}^{2+}$ with using the proposed sensor as an indicator electrode

The practical utility of the proposed membrane sensor was tested by its use as an indicator electrode for the titration of 25.0 mL of  $1.0 \times 10^{-4}$  mol L<sup>-1</sup> of  $\text{Co}^{2+}$  solution with EDTA  $1.0 \times 10^{-2}$  mol L<sup>-1</sup>. The resulting titration curve is shown in Fig. 10. As can be seen, the amount of  $\text{Co}^{2+}$  ions in solution can be accurately determined with the electrode.

### 3.12.2. Determination of Vitamin B<sub>12</sub> in Pharmaceutical Samples

As noted in the past, vitamin B<sub>12</sub> is a chelate with a central cobalt atom. The molar ratio of ligand to the central atom (cobalt) is 1:1 in this vitamin. For this reason, vitamin B<sub>12</sub> sublingual tablets and its injectable products were used as real samples. Due to fact that the proposed sensor is only susceptible to  $\text{Co}^{2+}$ , it was necessary to initially decompose this vitamin and convert it into free  $\text{Co}^{2+}$  ions and the sample treatment process was also carried out for the same reason, as well as for the destruction of all organic components and other substances present in the drug matrix [9]. The  $\text{Co}^{2+}$  concentration is easily determined by drawing the calibration curve and extrapolating the recorded potential from solution obtained after the treatment procedure. By using stoichiometry, vitamin B<sub>12</sub> assay can be determined indirectly in pharmaceutical products from the concentration of  $\text{Co}^{2+}$  solution easily.

**Table 9.** Determination results of vitamin B<sub>12</sub> in pharmaceutical samples

Real sample	Stated Content of vitamin B <sub>12</sub> (mg)	Found amount of vitamin B <sub>12</sub> (mg)	measured $\text{Co}^{2+}$ concentration (mol L <sup>-1</sup> )
Ravit-B ampoule	1	1.01(±0.020) <sup>a</sup>	$2.9776 \times 10^{-5}$
Vitamin B <sub>12</sub> ampoule	1	0.99(±0.015) <sup>a</sup>	$2.9304 \times 10^{-5}$
Vitamin B <sub>12</sub> sublingual pill	1	1.02(±0.018) <sup>a</sup>	$3.0024 \times 10^{-5}$

<sup>a</sup> %RSD based on three replicate analysis

At first, Ravit-B and vitamin B<sub>12</sub> ampoules were studied. Both ampoules each contain 1000 µg of cyanocobalamin and the molecular weight of cyanocobalamin is 1335.38 g mol<sup>-1</sup>. The  $\text{Co}^{2+}$  concentration was easily determined by drawing the calibration curve and extrapolating the recorded potential from solution obtained after the treatment procedure. By using stoichiometry, cyanocobalamin assay was determined indirectly in pharmaceutical

products from the concentration of  $\text{Co}^{2+}$  solution easily. The results are summarized in table 9.

The sublingual tablet of vitamin  $\text{B}_{12}$ , which contains 1000  $\mu\text{g}$  of methylcobalamin, was also studied. The molecular weight of methylcobalamin is 1344.40  $\text{g mol}^{-1}$ . The results are summarized in table 9, too. As shown in table 9, the proposed sensor has been able to determine the amount of this drug with a great precision and accuracy in two of its most common forms.

### 3.13. Comparison of the proposed $\text{Co}^{2+}$ sensor and some of the best previously reports

The Nernstian slope, linearity range, working pH range, life time and response time of proposed  $\text{Co}^{2+}$  sensor with some of the best previously reports are compared in Table 10.

**Table 10.** Comparison of the proposed  $\text{Co}^{2+}$  ion sensor and the some previously reports

Ionophore	Slope	Linear range (M)	Response time (S)	pH	Life time (month)	Ref.
cobalt (II) - 4- Formyl azohydrozoaniline antipyrine	29.0	$1 \times 10^{-6} - 1 \times 10^{-2}$	10	4.5-8.5	2	[43]
cobalt(II) complex of 2,4-dimethyl-1,5,8,11-tetraazacyclotrideca-1,4-diene [Me <sub>2</sub> (13)dieneN <sub>4</sub> ]	29.0	$7.94 \times 10^{-6} - 1 \times 10^{-1}$	10	2.5-7.0	3	[44]
9-t-Butyl-3,9,15,21-tetraaza-4,5,13,14-dibenzo-6,12-dioxabicyclo[15.3.1]hencosa-1(21),17,19-triene-2,16-dione	29.4	$2 \times 10^{-6} - 1 \times 10^{-2}$	10	3.0-8.0	2	[45]
4-tert-butylthiacalix[4]arene	30	$5.3 \times 10^{-6} - 1 \times 10^{-1}$	10	2.5-6.0	4	[46]
2,3,4-pyridine-1,3,5,8,11,14-hexaazacyclohexadeca-2-ene	30	$6.3 \times 10^{-6} - 1 \times 10^{-1}$	15	2.5-6.5	4	[47]
palladium(II) dichloro acetylthiophene fenchone azine(I)	29.6	$1 \times 10^{-6} - 1 \times 10^{-1}$	25	3.0-7.0	4	[48]
p-(4-n-butylphenylazo)calix[4]arene (I)	29.0	$9.2 \times 10^{-6} - 1 \times 10^{-1}$	25	4.0-7.2	3	[49]
Oxime of 1-(2-oxocyclohexyl)-1,2-cyclohexaediol	29.8	$1 \times 10^{-6} - 1 \times 10^{-1}$	25	3.5-8.0	2	[50]
1-(6-chloroquinoxaline-2-yl)hydrazine	29.4	$3 \times 10^{-6} - 1 \times 10^{-1}$	10	3.0-8.0	3	This work

As is obvious, the results clearly indicate that the proposed sensor is superior to some of the previously reported ones in terms of response time and working pH range. In linearity range, life time and Nernstian slope, proposed sensor is closed to the best previously reports.

#### 4. CONCLUSION

Vitamin B<sub>12</sub> is an essential and vital nutrition for humans and other living organisms that is used in many biological processes as well as in the treatment of certain diseases. A low-cost, easy-to-use, fast and reliable device is required for monitoring of this drug in pharmaceutical products. In this study, the Co<sup>2+</sup> potentiometric membrane sensor was constructed by 1-(6-chloroquinoxaline-2-yl) hydrazine as a new ionophore. Applicable pH range (3.0-8.0), fast response time (~10s), low detection limit ( $1.0 \times 10^{-6}$  M), long life time (12 weeks) and potentiometric selectivity coefficients of the proposed sensor make it a very good device used for the determinations of Co<sup>2+</sup> ions. It was used as an indicator electrode in potentiometric titration of Co<sup>2+</sup> with EDTA and in indirect determination of Vitamin B<sub>12</sub> by measurement of the cobalt ions in dietary supplements. Also, the various theoretical thermodynamic properties (such as enthalpy, entropy, Gibbs free energy and equilibrium constant) of the reaction complexation of some ions with sensing molecule were studied. The theoretical results in gas phase were shown the thermodynamic reactivity of complexation of copper ion with studied ligand is the best but in liquid phase according to experimental results were shown that the thermodynamic reactivity of complexation of cobalt ion with studied ligand is the best.

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