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Determination of Chromium(III) and Magnesium(II) Ions in Pharmacological and Real Water Samples using Potentiometric Sensors based on Chitosan Schiff base Derivative as Green and Sensitive Ionophore

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Abstract- In the study, novel and sensitive carbon paste electrodes (CPEs) developed for the potentiometric measurement of Cr(III) and Mg(II) ions in pharmacological and water samples. CPEs as indicator electrodes were prepared from a mixture of four components, including graphite powder, paraffin oil, multi-walled carbon nanotubes (MWCNTs), and a green ionophore (Chitosan Schiff base derivative). Optimization of CPE composites indicated that the critical factor in the preparation of the CPEs was chitosan Schiff base derivative which selectivity proposed sensors highly depended on this factor. Effects of various parameters such as pH, lifetime, response time, and selectivity of the potentiometric sensors to determine Cr(III) and Mg(II) ions were also evaluated. The results indicated that the proposed sensors can be applied in a relatively wide range of pH, long lifetimes, short response times, and excellent selectivity for the analyte determination. The prepared sensors show Nernstian slopes of 19.53 and 29.83 mVdecade^{-1}, and LODs of 5.6×10^{-8} and 4.4×10^{-9} with RSDs lower than 2.3% for Cr(III) and Mg(II) ion determination, respectively. The calibration curves were linear in the concentration ranges of 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-3} M, and R-square of 0.989 and 0.987 for the measurement of Cr(III) and Mg(II) ions, respectively. Besides, the sensors were successfully utilized for the Cr(III) and Mg(II) ion measurement in real samples such as tablet and tap water samples with suitable recovery higher than 97.6%.

Keywords- Potentiometric sensor, Chitosan Schiff base derivative, Cr(III) ion determination, Mg(II) ion measurement, Pharmacological sample, Tap water sample

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1. INTRODUCTION

Chromium(III) and magnesium ions are considered as two essential elements in the functioning of living organisms. Cr(III) ion enhances the insulin action in insulin-sensitive tissues [1]. This ion regulates glucose homeostasis by improving the activation of receptors of insulin through the oligopeptide uromodulin; as a result, sensitivity and transduction of insulin signal are increased by enhancing glucose uptake [1,2]. Dietary supplements containing chromium ions are also decreased total cholesterol and triglyceride levels in diabetic patients' blood [3]. Chromium(III) ion deficiency can lead to intolerance of glucose, blood circulating insulin elevation, impaired growth and, fasting hyperglycemia [4]. Mg(II) ion acts as a cofactor in most of 325 the enzymatic reactions in the human body [5,6]. This ion has antinociceptive effects in cases of chronic (neuropathic) and acute (postoperative) pain [7]. A poor diet of Mg(II) ion causes problems such as type II diabetes, cardiovascular disease, depression, and hypertension [8,9]. Usage of Mg(II) supplementation in clinical samples has improved blood pressure, cardiovascular system, metabolic syndrome, immune function, and clinical signs of mania [10-13]. However, the determination of these ions in real samples such as dietary supplements, human urine and plasma is essential to investigate their effects on human health. Therefore, providing new and inexpensive determination methods with suitable sensitivity and high accuracy can assist researchers to better evaluate these effects.

Electrochemical methods have been widely regarded because of their unique features such as ease of operation, low cost, high sensitivity, good reproducibility, ease of automation, and the ability to measure chemical species [14-16]. Among them, potentiometric methods do not require sophisticated analytical tools and are very inexpensive and straightforward with suitable sensitivity, long-term stability and high repeatability [17-19]. Besides, these methods can be applied for many real samples with complex, turbid, viscous, and color matrices without the need for a sample preparation step, especially for aqueous samples [20]. Potentiometric sensors usually prepared from two primary components, including an indicator electrode and a reference electrode in which sensitivity and selectivity of the potentiometric sensor are highly depended on type and structure indicator electrode. Carbon paste electrode (CPEs) is widely utilized as proper indicator electrode because of unique properties such as low ohmic resistance, renewability, ease of its preparation and structure modification, stable response, and free- internal solution. Traditional CPEs for determination of metal ions are usually prepared from a mixture of graphite powders, paraffin oil as a binder, and a suitable ligand as an ionophore. Essential properties of graphite powder in CPEs are high chemical purity, proper electrical conductivity, suitable particle size and uniform distribution of the particles [21]. However, usage of carbon-based nanomaterials as an additive nanomaterial to the CPE components can modify surface electrode with enhancing the surface-to-volume ratio of the electrode, mechanical and chemical stability, and electrical conductivity [22,23]. Selecting suitable ligand (ionophore) is a critical factor in the preparation of CPE that has significant effects in the selectivity and sensitivity of CPE to determine the analyte based on the type of its interaction with the analyte. Therefore, there is a strong tendency for synthesis of new and green ligands to interact selectively with the analyte for the CPE development to measure the analyte in samples with complex matrices.

Chitosan is a polysaccharide polymer with a natural origin which is used in many applications such as pharmaceutical drug delivery [24], removal of heavy metal ions [25], biomedical and pharmaceutical applications [26], packaging material [27], and etc. due to its unique features including less toxic, biodegradable, and biocompatible [28]. The presence of amine and hydroxyl groups in chitosan can interact well with different materials in optimal conditions. Also, these functional groups can be easily functionalized with other groups to synthesize chitosan derivatives. Obviously, the synthesis of chitosan derivatives with appropriate functional groups can improve its interaction with analyte and sensor selectivity.

In the work, novel and sensitive potentiometric sensors were developed to measure Cr(III) and Mg(II) ions in pharmacological and water samples. CPEs as indicator electrodes were prepared from a mixture of four components, including graphite powder, paraffin oil, multi-walled carbon nanotubes, and a green ionophore (chitosan Schiff base derivative). Effects of various parameters such as pH, lifetime, response time, and selectivity of the potentiometric sensors to determine Cr(III) and Mg(II) ions were evaluated. The results indicated that the proposed sensors can be applied in a relatively wide range of pH, long lifetimes, short response times, and excellent selectivity for the analyte determination. Besides, the sensors were successfully utilized for the Cr(III) and Mg(II) ion measurement in real samples such as tablet and tap water samples.

2. EXPERIMENTAL

2.1. Chemical and instruments

Acetic acid, 4-Chlorobenzaldehyde (purity 97%), sodium hydroxide, ethanol (HPLC grade), and hydrochloric acid (purity 37%) were purchased Merck (Darmstadt, Germany) and used to synthesize Chitosan Schiff base derivative without each purification methods. Chitosan low molecular weight and MWCNTs (>95% purity and OD 20-30 nm) were obtained from the Sigma-Aldrich (USA) and US Research Nanomaterials, Inc, respectively. Graphite powder (purity 99%, mesh 325), Chromium(III) nitrat*e* nonahydrate (purity≥99.99), and Magnesium nitrate hexahydrate (purity 99.99) were purchased Merck (Darmstadt, Germany). Standard solutions of Cr(III) and Mg(II) ions (1.0×10^{-1} M) were prepared by dissolving a proper amount of $Cr(NO_3)_3.9H_2O$ or $Mg(NO_3)_2.9H_2O$ in distilled water.

A pH/mV meter (Metrohm-691, Switzerland) was utilized to measure the sensor potential and adjust the solution pH. The CPEs and an Ag/AgCl (Azar electrode, Iran) were chosen as the indicator electrode and the reference electrode to determine Cr(III) and Mg(II) ions in sample solutions, respectively. The following potentiometric cell was applied to determine EMFs with the sensors at room temperature (25±1 °C).

Ag, AgCl, KCl (satd.) || analyte sample solution | the CPEs

2.2. Ligand synthesis

The green ligand (Chitosan Schiff base derivative) was prepared using the previous article[29]. Briefly, a solution of chitosan (1.0 g) in 50.0 mL of acetic acid (2% w/v) was prepared by stirring at room temperature for 6 h. Then, 4-chloro benzaldehyde (1.86 mM) in 10.0 mL of ethanol was added dropwise to the chitosan solution. The obtained mixture was then stirred at 50 °C for 6 h. Sodium hydroxide solution (5% w/v) was added to precipitate the chitosan Schiff base. The obtained precipitation was washed twice with water and twice with ethanol to remove residual reactants, respectively. The precipitation was finally dried using a vacuum oven at 60 °C for 16 h. The chemical structure of Chitosan Schiff base derivative was represented in Fig. 1.

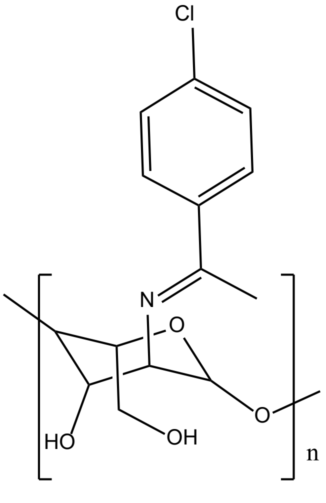


Fig. 1. Chemical structure of chitosan Schiff base derivative

2.3. Carbon paste electrodes preparation

The proposed potentiometric sensors for the determination of Cr(III) and Mg(II) ions were prepared by mixing precise amounts of graphite powder, paraffin oil, MWCNTs, and Chitosan Schiff base derivative in a mortar for 25 min. The optimum amounts of each electrode component are presented in Table 1. The obtained paste was firmly packed into a glass tube with an internal diameter of 2.8 mm and a length of 3.0 cm to prevent the formation of air bubbles within the electrodes, which usually cause an increase the electrode resistance and a decreasing its performance. A copper wire is carefully inserted into the paste inside the electrode to provide the electrical contact of the electrode. To create a new surface of the electrode, the old surface of the electrode was removed and the new carbon paste was replaced. The new surface of the electrode was finally conditioned by placing the electrode in Cr(III) or Mg(II) ions solution at a concentration of 1.0×10^{-2} M for 20 h before being used to measure these ions in the sample solution.

3. RESULTS AND DISCUSSION

3.1. Optimization of components of CPEs

The performance and sensitivity of CPEs generally depend on the nature of its components and the ratio between them. Therefore, the amount of CPE components, including graphite powder, MWCNTs, paraffin oil, and Chitosan Schiff base derivative, were investigated by preparing several CPEs and determining the slope obtained for each analyte. The results of Initial investigation of the cations which could interact with the fabricated electrodes were shown, Cr(III) and Mg (II) have the appropriate slope and then were selected for were utilized for further study. The amounts of each component of the electrode and the slope values obtained for them are presented in Table 1.

Table 1. Percentage of CPE components and slope values obtained for determination of Cr(III) and Mg(II) ions

CPEs No.	Graphite powder	MWCNTs	Paraffin oil	Ligand	Slope for Cr^{3+} mVdecade^{-1}	R^2	Slope for Mg^{2+} mVdecade^{-1}	R^2
1	37	18.9	25.5	18.9	17.51±0.21	0.953	28.29±0.23	0.975
2	37	17.5	28	17.5	16.92±0.18	0.928	26.57±0.24	0.956
3	37	16.1	28.7	18.2	16.51±0.24	0.936	27.71±0.19	0.983
4	37	14.7	29.4	18.9	15.17±0.20	0.924	28.42±0.21	0.961
5	37	18.9	29.4	14.7	14.23±0.27	0.908	25.07±0.23	0.954
6	37	18.2	26.6	18.2	-17.25±0.19	0.976	29.20±0.18	0.972
7	**37**	**18.2**	**28.7**	**16.1**	**-19.53±0.17**	**0.989**	26.78±0.22	0.976
8	50	15	20	15	15.92±0.21	0.932	29.02±0.19	0.985
9	**60**	**10**	**20**	**10**	-14.36±0.25	0.916	**29.83±0.16**	**0.991**
10	60	0	20	20	-13.72±0.23	0.921	27.25±0.24	0.972
11	60	18.2	21.8	0	-9.31±0.22	0.926	14.17±0.21	0.943

Optimized values of each sensor are text bold

The results indicated that the ligand amount has a significant effect on the obtained slopes for determining Cr(III) and Mg(II) ions, which the obtained slope profoundly changes with changing this factor. It may be due to generate the suitable interactions between analytes and functional groups on the chitosan Schiff base derivative as the ligand in the CPEs. Therefore, the best slopes were obtained for 16.1 and 10.0 wt.% of the ligand for Cr(III) and Mg(II) ions, respectively. Also, increasing MWCNTs in the CPE compositions can be modified the CPEs by increasing electrode surface area and enhancing the conductivity of the CPE surface [30]. The values of MWCNTs of 18.2 and 10.0 wt.% were chosen as the optimal values to prepare the CPEs for the determination of Cr(III) and Mg(II) ions, respectively. According to Table 1, the electrodes (No. 7 and 9) with slopes of 19.53 and 29.84 mVdecade^{-1} were selected as the optimal ingredient composition of the CPEs for the Cr(III) and Mg(II) ion measurement, respectively. These CPE compositions were utilized for further study.

3.2. pH effect on the CPEs response

A critical parameter with a high effect on the performance of a potentiometric sensor for determination of metal ion is the pH of the sample solution. The parameter can affect the interaction of analyte and electrode surface with changing the charge of ligand functional groups on the electrode and metal ion species in the sample solution. Therefore, the pH effects on the CPEs responses for the determination of Cr(III) and Mg(II) were investigated using the prepared CPEs presented in Table 1, no. 7 and 9, respectively. The concentration of Cr(III) or Mg(II) ion in the sample solution is fixed at 1.00×10^{-3} M, while the pH of the sample solution is changed using nitric acid (0.1 M) or sodium hydroxide solution (0.1 M) in the range of 4-9.5 and 2-12 for the determination of Cr(III) and Mg(II) ions, respectively (Fig. 2). According to Fig. 1, the potentials of the sensors remain constant in the ranges of 5.5-9.0 and 3.5-10.0 for the determination of Cr(III) and Mg(II), respectively. At low pH, the possibility of proper interaction between the cations and the electrode is reduced by changing the ligand functional groups present in the electrode.

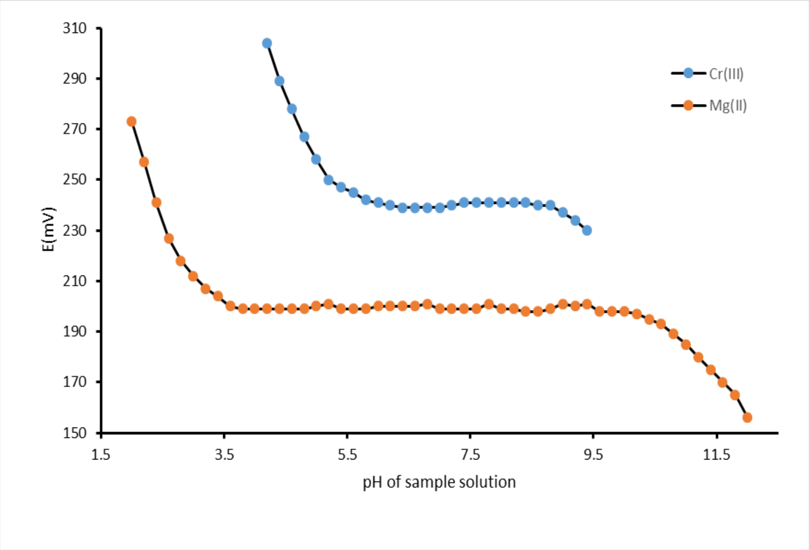


Fig. 2. Effect of pH of sample solution on the potential of the Cr(III) and Mg(II) sensors with a concentration of 1×10^{-3} M

In other words, the hydroxyl groups of the ligand interact with the hydrogen ions at low pH and create a positive charge on the ligand that prevents the analyte from interacting with the ligand due to electrical repulsion.

At high pH of the sample solution, Cr(III) and Mg(II) ions precipitate as $Cr(OH)_3$ and $Mg(OH)_2$ or other species; as a result, the electrode potential changes. Thus, these sensors can utilize thoroughly in the pH ranges of 5.5-9.0 and 3.5-10.0 for the measurement of Cr(III) and Mg(II), respectively.

3.3. Sensor response time and lifetime

The response time of the CPEs is defined as the mean time required to reach the potential of CPE within ±0.1 mV of the final equilibrium potential, after consecutive immersion in Cr(III) or Mg(II) solution in the concentration ranges of 1.0×10^{-8} to 1.0×10^{-2} or 1.0×10^{-8} to 1.0×10^{-3}, respectively [31].

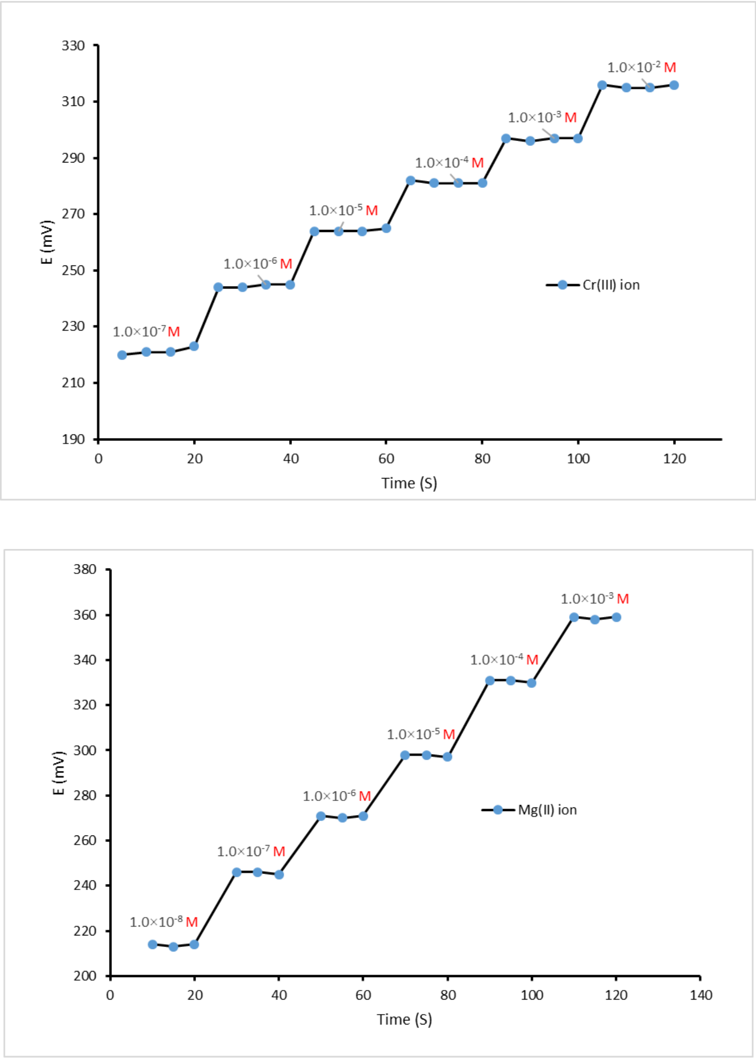


Fig. 3. Response times of the sensors for the determination of Cr(III) and Mg(II) ions

The variation curve of the electrode potential versus time for the Cr(III) and Mg(II) ion measurements is presented in Fig. 3, indicating the proposed sensors have the response time of 5 and 10 s for the determination of Cr(III) and Mg(II) ions, respectively. Therefore, the response time of CPEs for determination of Cr(III) ion is lower than the response time of CPEs for determination of Mg(II) ion, as a result, the interaction kinetics of the chitosan Schiff base derivative as the ligand in the CPEs with Cr(III) ion is faster than Mg(II) ion.

The sensor stability for the determination of analyte can be evaluated by investigating the electrode lifetime. For this purpose, the slope of the calibration curve was weekly determined for measuring the standard solution of Cr(III) or Mg(II) ions using the proposed CPEs under optimum conditions (Fig. 4). Both sensors designed to measure Cr(III) and Mg(II) ions showed a lifetime of more than 8 weeks with slopes of 19.34±0.19 and 29.48±0.27 mVdecade^{-1}, respectively, and without any significant deviation from the Nernstian behavior. For the longer lifetime, a decrease in the slope obtained with these electrodes was observed, which may be due to the instability of the chitosan Schiff base derivative and its chemical structure degradation by temperature.

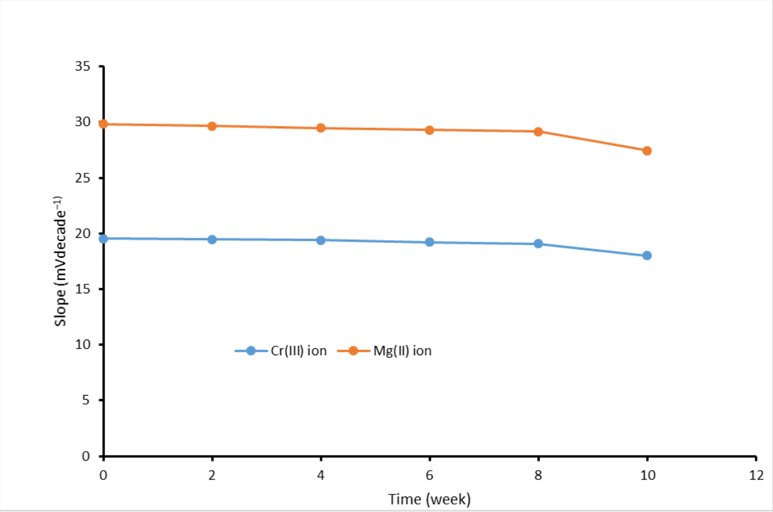


Fig. 4. The lifetimes of the sensors for the determination of Cr(III) and Mg(II) ions

3.4. Sensor selectivity

One of the critical factors that can affect the potential of the proposed sensors for analytes is the species present in the real sample matrix which the sensor application for the analyte measurement in real samples relies on highly them. The selectivity coefficients were calculated for the Cr(III) or Mg(II) sensor based on the matched potential method (MPM). The selectivity coefficient (K_{MPM}) was determined using the Eq.1.

$$K^{MPM}_{Metal\ ion,X} = \frac{a_{metal\ ion}}{a_X} \qquad (1)$$

where $a_{Metal\ ion}$, $K^{MPM}_{Metal\ ion,X}$, $and\ a_X$ are the activity of Cr(III) or Mg(II) ion, the selectivity coefficient, and interfering ion, respectively. To obtain the selectivity coefficient, the concentration of Cr(III) or Mg(II) ions of 1.0×10^{-4} M was selected while the concentration of interfering ions was changed in the range of 1.0×10^{-5}-1.0×10^{-1} M. The obtained selectivity coefficients were represented in Table 2, indicating the proposed sensors have suitable selectivity for the determination of Cr(III) and Mg(II) ions and can be applied well for their analysis in real samples.

Table 2. Selectivity coefficients (K_{MPM}) of various species for the determination of Cr(III) and Mg(II) ions with the CPE sensors

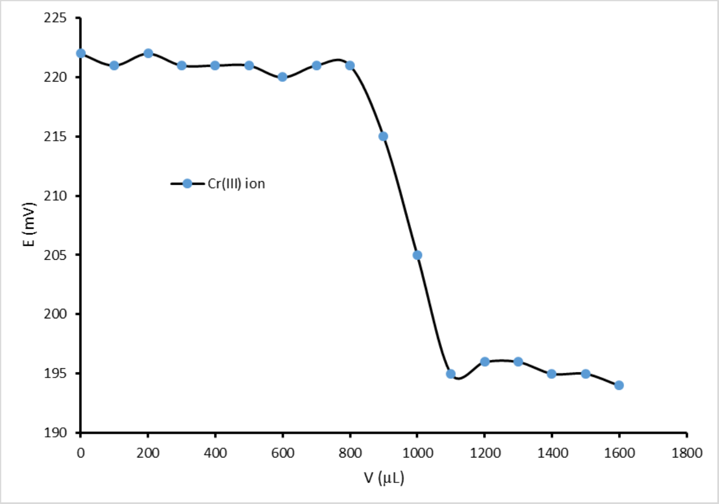
Interference species	**K_{MPM} ×10^{-3}**	
	Cr(III) determination	Mg(II) determination
Cd^{2+}	0.81	9.0
Pb^{2+}	1.0	6.5
Ni^{2+}	7.1	0.49
Zn^{2+}	0.62	2.15
Li^{+}	4.62	1.1
NH_4^{+}	0.84	0.91
Cr^{3+}	----	68
Mg^{2+}	2.04	----

3.5. Analytical performance

The figure of merits of the proposed sensors were determined by investigating the linearity, limit of quantitation, limit of detection, and relative standard deviation for measurement of Cr(III) and Mg(II) ions. Under the optimal conditions, good linearity in the range of 1.69×10^{-7}–1.00×10^{-2} and 1.33×10^{-8}–1.00×10^{-3} M with suitable R square higher than 0.99 and 0.98 were obtained for Cr(III) and Mg(II) ion determination in the water sample, respectively. Limit of detection and limit of quantitation was calculated $3S_b/m$ and $10S_b/m$, where S_b and m are the standard deviations for five replicate the blank sample determinations and slope of the calibration curve, respectively. LODs and LOQs were lower than 5.60×10^{-8} and 1.69×10^{-7} M for Cr(III) and Mg(II) ion determinations, respectively. The method precision was investigated by determining the relative standard deviation (RSDs) for five replicate the measurement of the standard solution of Cr(III) or Mg(II) ions at a concentration of 1.00×10^{-4}M. The RSDs were less than 2.3% for both analytes. The obtained results were presented in Table 3.

Table 3. The figure of merits of the proposed methods for Cr(III) and Mg(II) ion determination with the CPEs sensors

Analyte	**Cr(III) ion**	**Mg(II) ion**
Calibration equation	E(mV)=19.529×C(M)+225.61	E(mV)=29.827×C(M) +150.07
R-squared	0.989	0.987
Linearity (M)	1.69×10^{-7}-1.00×10^{-2}	1.33×10^{-8}-1.00×10^{-3}
Limit of detection	5.60×10^{-8}	4.40×10^{-9}
Limit of quantitation	1.69×10^{-7}	1.33×10^{-8}
Relative standard deviation (n=5)	2.3%	2.1%



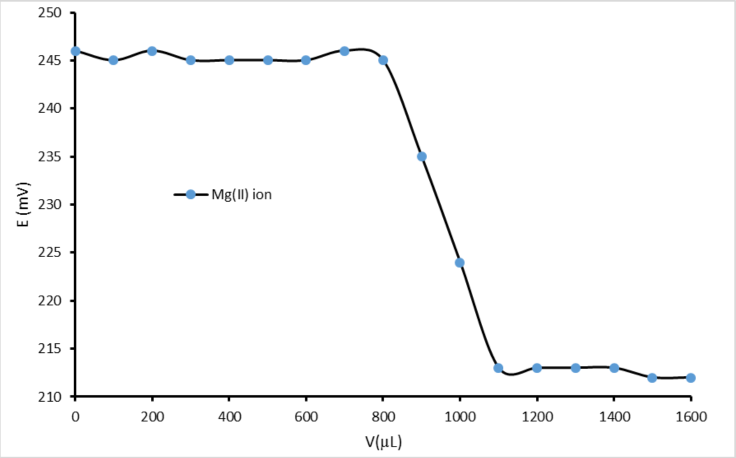


Fig. 5. The potentiometric titrations 10.0 mL of 1.0×10^{-2} M of Cr(III) or Mg(II) ions with EDTA solution (1.0×10^{-2} M)

3.6. Real sample assay

The proposed sensors as indicator electrodes were used in the potentiometric titrations to evaluate their applicability. An EDTA solution (1.0×10^{-2} M) was selected as a titrant and added to a 10.0 mL of 1.0×10^{-2} M of Cr(III) or Mg(II) ions to draw the titration curves (Fig. 5). The results demonstrated good titration curves with excellent stoichiometry for both analytes. The potential of the sensors changed logarithmically before the endpoint of the titration curve and remained almost constant with the further increase in titrant due to the low activity of the analyte ions in the sample solution. The average endpoints of 9.89 and 9.86 mL with RSDs of 1.1 and 1.3% were calculated from three replications of titration procedures for the determination of Cr(III) and Mg(II) ions, respectively. As a result, the concentration of Cr(III) and Mg(II) ions of 9.89×10^{-4} and 9.86×10^{-4} M were obtained using the average endpoints, respectively.

The ability of the sensors to measure the analytes in real samples, including pharmaceutical and water samples, was investigated. DiaFit capsule (EuRho VITAL, Germany) was obtained from a drugstore in Mashhad, Iran. The contents of a capsule were carefully transferred into a vial. The concentrated hydrochloric acid (3.0 mL) was added to it. The obtained solution was then diluted to 25.0 mL with distilled water. Finally, the solution was spiked with the standard solution of Cr(III) or Mg(II) ions to determine Cr(III) and Mg(II) ions by the proposed sensors. Tap water samples were obtained from Mashhad, Iran. The tap water samples were centrifuged for 10 min at 8,000 rpm and filtered before spiking with standard solutions of Cr(III) or Mg(II) ions. The amounts of standard solution of the analytes added to the real samples, obtained concentrations of the analytes, and the calculated recoveries were presented in Table 4, conforming the proposed sensors are suitable for the measurement of both analytes in the water and pharmaceutical samples with significant recovery (≥97.6) and RSDs (≤3.3%).

Table 4. Determination of Cr(III) and Mg(II) in tablet and tap water samples with the proposed sensors

Sample	**Added (mgL^{-1})**	**Cr(III)**		**Added (mgL^{-1})**	**Mg(II)**	
		Found(M)	Recovery (%)		Found(M)	Recovery (%)
Tablet	0.0	$4.60(\pm0.15)\times10^{-4}$	----	0.0	$5.21(\pm0.17)\times10^{-3}$	----
	1.0×10^{-4}	$5.48(\pm0.17)\times10^{-4}$	97.8	1.0×10^{-3}	$6.06(\pm0.19)\times10^{-3}$	97.6
	5.0×10^{-4}	$9.43(\pm0.28)\times10^{-4}$	98.2	5.0×10^{-3}	$1.04(\pm0.03)\times10^{-2}$	101.9
Tap water	0.0	$8.39(\pm0.23)\times10^{-7}$	----	0.0	$5.45(\pm0.16)\times10^{-4}$	----
	1.0×10^{-6}	$1.89(\pm0.05)\times10^{-6}$	102.8	1.0×10^{-3}	$1.51(\pm0.05)\times10^{-3}$	97.7
	5.0×10^{-6}	$5.73(\pm0.14)\times10^{-6}$	98.1	5.0×10^{-3}	$5.43(\pm0.12)\times10^{-3}$	97.9

4. CONCLUSION

The potentiometric sensors based on carbon paste electrodes (CPEs) as indicator electrodes were developed for the measurement of Cr(III) and Mg(II) ions in pharmacological and water samples. Chitosan Schiff base derivative as a green ionophore was prepared and applied in the CPEs. Usage of Chitosan Schiff base derivative in the CPE preparation has been enhanced the selectivity of sensors to determine the analytes. Besides, MWCNTs as another component in the CPEs can be increased the electrode surface and modified mechanical and chemical stability, and electrical conductivity. The advantages of the proposed sensors for the determination of Cr(III) and Mg(II) ions are Cheap, simple operation, long lifetimes, short response times, and excellent selectivity. The sensors are also used in the wide range of the pH of the sample solution. Application of the sensors in the real sample including tap water and tablet samples confirmed that the proposed sensors are suitable for daily measurement of the analytes with high recovery and low RSDs for medical diagnostics and water quality control laboratories.

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