

6-Ketomethyl Phenanthridine as a New Carrier in the Construction of a Highly Selective Fe(III) Ion-Selective Membrane Electrode

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A new Fe³⁺ ion-selective membrane electrode based on 6-ketomethyl phenanthridine (6-KMPT) and 1-(4-dimethyl aminophenyl)-2-(5H-phenanthridine-6-ylidene)-ethanone, which were incorporated in a plasticized polyvinyl chloride (PVC) membrane, is described. The optimized membrane demonstrated a linear dynamic range of 5.2×10^{-6} - 1.0×10^{-2} M, with a near Nernstian slope of 19.5 ± 1.5 mV per decade and a detection limit of 1.2×10^{-6} M. The electrode showed high selectivity for Fe³⁺ ions in comparison to other metal ions. The effect of membrane composition on the potential response of the electrode was studied. The best performance was observed for the membrane comprised of electroactive material (6-KMPT), dioctyl phthalate (DOP) as plasticizer, and PVC in the optimum ratio of 5:67:28% w/w. The pH working range of the sensor was 1.2-3.0. The proposed Fe³⁺ sensor was successfully used for determining the presence of iron in pharmaceuticals and the results were in good agreement with the value obtained using the atomic absorption spectroscopic method.

Key Words: 1-(4-Dimethyl aminophenyl)-2-(5H-phenanthridine-6-ylidene)-ethanone, Fe(III) ion-selective electrode, PVC membrane.

Introduction

Iron is widely distributed in nature and is among the most important elements in biological systems. It plays an important role in oxygen and electron transport, provides a fundamental structure for hemoglobin, heme

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enzymes, and many co-factors involved in enzyme activity. Moreover, it is safe to say that, with only a few possible exceptions in the bacterial world, there would be no life without iron.¹ The absence of iron in an organism causes anemia, the result of a decrease in red blood cell content. This deficiency is treated with iron salts via oral or intramuscular administration;² however, if the concentration of iron exceeds the normal level it may become a potential health hazard. The treatment of excess iron with iron salts may produce severe poisoning, resulting in symptoms of gastric irritation, vomiting, pallor, and circulatory collapse.² Hence, the development of new and simple methods for the determination of iron in clinical, medicinal, environmental, and different industrial samples is of great interest. Ion-selective electrodes have found a wide range of use in diverse fields of analysis because they are cost effective, selective, sensitive, and applicable over a wide range of experimental conditions.^{3,4} In addition, the successful application of potentiometric titration and direct potentiometry in routine chemical, environmental, and clinical analysis has stimulated a search for ionophores that can chemically recognize specific ions and offer either new or improved selectivity for different ions for developing ion-selective electrodes. During the past few decades a large number of ionophores, especially a wide variety of neutral macrocyclic polyethers based on nitrogen and sulfur, have been synthesized and widely used in potentiometric sensors for the determination of respective ions in environmental, industrial, and clinical samples.^{3,5} The most attractive features of potentiometry using ion-selective electrodes are the speed with which samples can be analyzed, portability of the device, sample non-destruction, online monitoring, cost effectiveness, and a wide measuring range.^{6,7} The reported number of sensors for iron are few;^{3,8-17} thus, direct potentiometry can be an advantageous alternative to those previously proposed.

In continuation of our research on the development of new ionophores for construction of ion-selective electrodes,¹⁸⁻²¹ the present work reports a new, highly selective iron PVC membrane electrode based on recently synthesized 1-(4-dimethyl aminophenyl)-2-(5H-phenanthridine-6-ylidene)-ethanone (Scheme 1, 2a) as an ionophore. Furthermore, the characteristic performance and analytical application of the proposed sensors were determined.

Experimental

Reagents

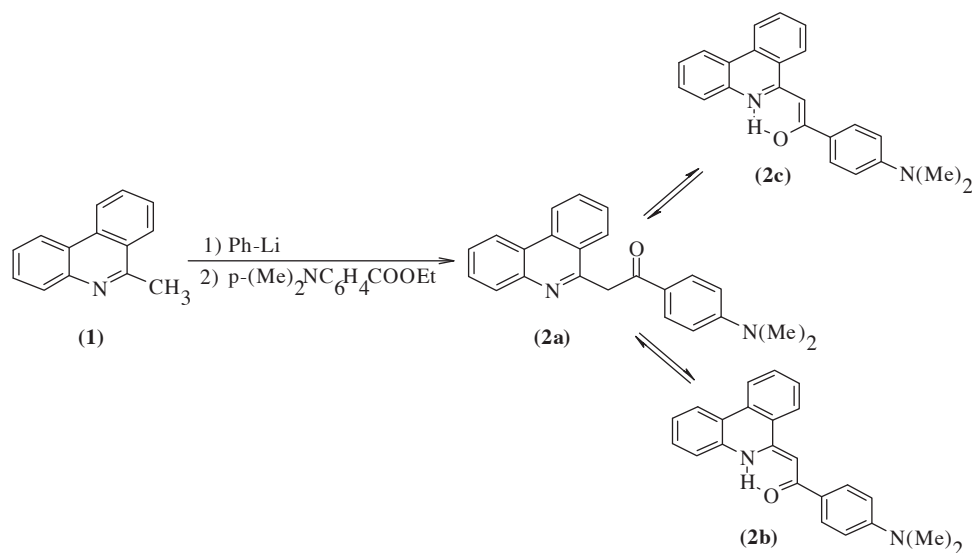
High molecular weight PVC powder, dibutyl phthalate (DBP), dioctyl phthalate (DOP), and 2-nitrophenyl octyl ether (NPOE) were obtained from Fluka. Tetrahydrofuran (THF) was obtained from LAB-SCAN. All of the metal nitrates and chloride salts (all from E. Merck) were of the highest purity available, and were used without further purification. 6-KMPT was synthesized, characterized, and purified at Isfahan University, Isfahan, Iran.

General Procedure for the Preparation of 6-KMPT

6-KMPT was prepared by the Bischler-Napieralski method;^{22,23} compound (2a) was prepared by the Goldberg and Levine method,^{24,25} purified via plate chromatography, and crystallized from ethanol as described below.

A 20-mL solution of 10 mmol of 6-methylphenanthridine (Scheme 1, 1) prepared in dry diethyl ether was added dropwise to a solution of phenyl lithium (12 mmol) in dry diethyl ether (20 mL). After stirring under an argon atmosphere for 30 min, 4-dimethyl amino-benzoic acid ethyl ester (10 mmol) in dry diethyl ether (20

mL) was added dropwise to the lithium derivative of 6-methylphenanthridine. The mixture was refluxed for 5-6 h. The resulting crude product was dissolved in cold water (30 mL), the organic layer was separated, and then it was dried with MgSO_4 and evaporated. Pure product was obtained by thin layer chromatography using n-hexane/ethyl acetate (6:1) as eluent (Scheme 1).



Scheme 1. Schematic preparation of 6-KMPT and its tautomerization.

Characterization of 1-(4-Dimethyl aminophenyl)-2-(5H-phenanthridine-6-ylidene)-ethanone (2b), (6-KMPT)

Yield: 65%; mp: 198-200 °C.

IR (KBr): 1600 cm^{-1} (C=O/C=C), 1550 cm^{-1} (C=C/C=O), and 1590 cm^{-1} (Ar).

MS (EI, 70 eV): m/z (%) = 340 (M^+ , 100), 148 ($p\text{-Me}_2\text{NC}_6\text{H}_4\text{CO}^+$, 75), and 121 ($p\text{-Me}_2\text{NC}_6\text{H}_5$, 40).

$^1\text{H-NMR}$ (500 MHz CDCl_3): δ = 3.10 (6 H, s, 2 CH_3), 6.83 (1 H, s, C=CH), 6.79-8.36 (12 H, m, $\text{C}_{13}\text{H}_8\text{N}$, C_6H_4), and 15.72 (1 H, s, NH).

$^{13}\text{C-NMR}$ (125 MHz CDCl_3): δ = 40.10 [$\text{N}(\text{CH}_3)_2$], 84.46 (vinyl-C), 110.98, 117.59, 119.79, 122.32, 122.42, 122.72, 124.81, 124.84, 127.81, 128.16, 128.73, 129.63, 131.43, 131.94, 135.13, 140.91, 152.15 (aryl-C), and 186.65 (C=O).

Apparatus

All potentiometric measurements were obtained with a Metrohm 744 pH meter (Switzerland) using the proposed sensor in conjunction with a double junction Ag/AgCl reference electrode (Azar Electrode Co.).

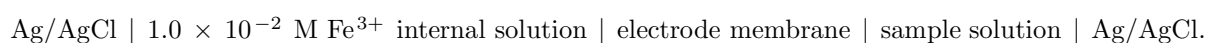
Atomic absorption measurements were performed with a Shimadzu Model AA 670 atomic absorption spectrophotometer (Japan) equipped with an air-acetylene burner, iron hollow cathode lamp ($\lambda = 248.3\text{ nm}$), and D_2 lamp for background correction. Absorption spectra were recorded on a Perkin Elmer model Lambda 25 (U.S.A.) UV-Vis spectrophotometer equipped with 10-mm quartz cells.

Preparation of the Membrane-Selective Electrode

The optimized membrane composition (KMPT 7.5 mg, DOP 97.5 mg, and PVC 45 mg) was dissolved in 5 mL of THF. The resulting solvent was evaporated slowly at room temperature until a viscose mixture was obtained. A Pyrex or Teflon tube (2-4 mm i.d.) was dipped into the mixture for about 10 s so that a membrane about 0.3 mm thick was formed. The tube was then pulled out of the mixture and kept at room temperature for about 12 h. The electrode tube was then filled with an internal filling 1.0×10^{-2} M Fe^{3+} solution and it was finally conditioned for 24 h by soaking in a 1.0×10^{-2} M solution of ferric chloride.

Electrode System and EMF measurements

Potentials were measured at 25 °C with the following cell compartment:



All measurements were carried out in a 25-mL glass cell with constant magnetic stirring of the test solution.

Results and Discussion

The structure of the compound used as the ionophore in the construction of the Fe^{3+} ion-selective electrode is presented in Scheme 1. The ligand was incorporated into the plasticized PVC membrane that defined the selectivity of the electrodes via selective complex formation with the Fe^{3+} cation of interest. Due to sufficient insolubility of 6-KMPT in water and the presence of donating nitrogen and oxygen atoms in its structure, it was expected to act as a suitable ion carrier in the PVC membranes, with respect to special transition and heavy metal ions of proper size and charge. Molecular absorption spectroscopy was used to confirm the interaction between the Fe^{3+} ions and 6-KMPT. The absorption spectra of 6-KMPT alone and in the presence of Fe^{3+} ions are shown in Figure 1. As can be seen, absorbance of the methanol solution of 6-KMPT at wavelengths of 428 and 454 nm decreased with the addition of Fe^{3+} ions to the solution. On the other hand, the spectral evolution observed involved the formation of a well-defined isobestic point at 330 nm, indicating the presence of one absorbing complex compound between 6-KMPT and the Fe^{3+} ions.

In preliminary experiments 6-KMPT was used as a neutral carrier to prepare PVC-based membrane electrodes for a variety of metal ions. The potential responses of the studied cation-selective electrodes, prepared under the same experimental conditions (except for 24 h of conditioning in a 1.0×10^{-2} M solution of the corresponding cations), are shown in Figure 2. As can be seen, among the cations tested in the concentration range of 1.0×10^{-5} - 1.0×10^{-2} M, the membrane sensor showed high selectivity for Fe^{3+} ions with a near-Nernstian behavior, probably due to a strong interaction between the ionophore and Fe^{3+} ions.

As the sensitivity and selectivity of an electrode depend significantly on membrane composition, the influences of the nature of the plasticizers, such as NPOE, DOP, and DBP, on the response characteristics of the Fe^{3+} ion-selective electrode at a constant quantity of 6-KMPT were investigated and the obtained results are summarized in Table 1. As can be seen, among the various plasticizers used, membranes containing 30% w/w PVC, 65% w/w DOP, and 5% w/w 6-KMPT had near-Nernstian behavior with a wide linear dynamic range.

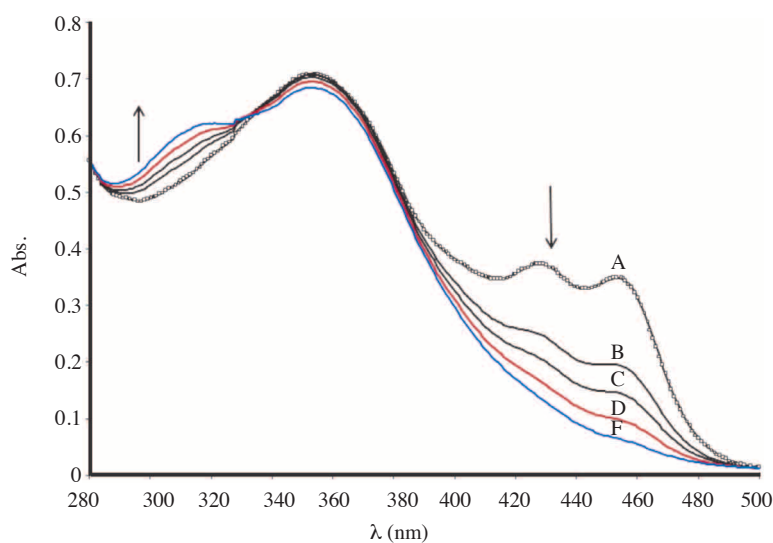


Figure 1. UV-Visible spectra of (A) 6-KMPT and (B-F) its complexes with different quantities of Fe^{3+} in methanol.

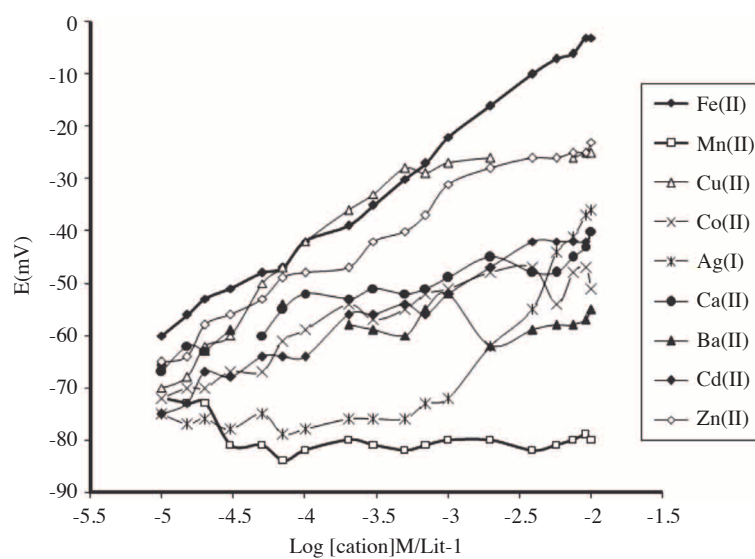
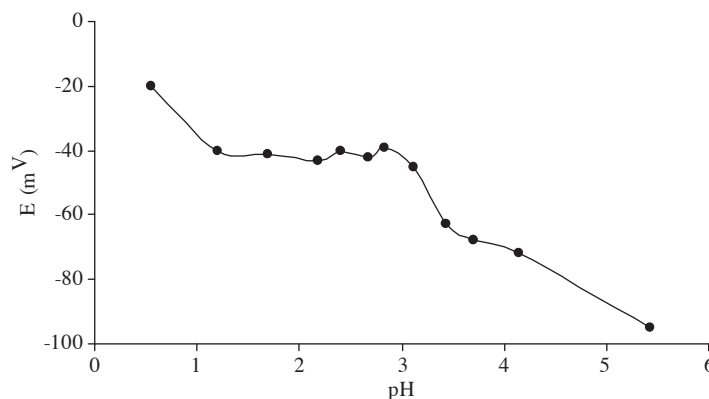


Figure 2. Potential response of the electrodes to various cations based on ligand 6-KMPT. The electrodes were conditioned in 1.0×10^{-2} M of the corresponding cation for 24 h.

The influence of pH on the response of the proposed sensors was evaluated by measuring the potential variation over a pH range of 0.5-5.5 for a solution of 1.0×10^{-4} M Fe^{3+} . The obtained results using the proposed sensor show that the electrode potential was independent of the solution's pH in the range of 1.2-3.0. Over this range the potential does not vary more than ± 1 mV (Figure 3). The effect of pH on the response characteristics at $\text{pH} > 3.0$ can be explained by the formation of an iron hydroxide precipitate. At pH levels < 1.2 the increase in potentials was most probably due to electrode response towards hydrogen ions.

Table 1. Optimization of membrane composition.

Components in membranes (%)				Slope (mVdec ⁻¹)	Response range (M)
PVC	6-KMPT	TPB	Plasticizer		
28	5	-	o-NPOE	14.76	$3.0 \times 10^{-4} - 1.0 \times 10^{-2}$
28	5	-	DOP	19.5	$5.2 \times 10^{-6} - 1.0 \times 10^{-2}$
28	5	1	DOP	15.45	$5.0 \times 10^{-5} - 1.0 \times 10^{-2}$
28	5	1	o-NPOE	13.63	$1.0 \times 10^{-4} - 1.5 \times 10^{-2}$
28	5	-	DBP	17.3	$8.0 \times 10^{-5} - 3.5 \times 10^{-2}$

**Figure 3.** Effect of pH on the potential response of the proposed Fe³⁺ ion-selective electrode.

For analytical applications the response time of a sensor is an important factor. The average time required for the Fe³⁺ ion-selective membrane electrode to reach a potential within ± 1 mV of the final equilibrium value after successive immersion of a series of Fe³⁺ ion solutions, each having a 10-fold difference in concentration, were determined. The static response time of the proposed PVC membrane sensor depended strongly on the concentration change. In the concentration range of 1.0×10^{-6} - 1.0×10^{-4} M the response time was 1-2 min, but when it ranged from 1.0×10^{-4} to 1.0×10^{-2} , the response time was < 40 s. The proposed membrane sensor was very stable and could be used over a period of 3 months without considerable change in its response characteristics.

The influence of the concentration of the internal solution on the potential response of the proposed sensor was studied. The Fe³⁺ concentration ranged from 1.0×10^{-4} M to 1.0×10^{-2} M and an EMF vs. $\log [\text{Fe}^{3+}]$ plot was determined. It was observed that variation in the concentration of the internal solution did not cause any significant difference in the potential response, except for an expected change in the intercept of the resulting plots. Due to the stability of the potentials and high sensitivity, a 1.0×10^{-2} M of Fe³⁺ solution was chosen as the internal solution concentration for further study.

Potentiometric selectivity coefficients are clearly the most important characteristics of a sensor and are especially critical for direct potentiometric measurements. The selectivity of the proposed electrode to other cations was investigated using the matched potential method (MPM).²⁶⁻²⁸ According to MPM, the selectivity coefficient is defined as the activity ratio of the primary ion (A) and the interfering ion (B) that gives the same potential change in a reference solution. Thus, one should measure the change in potential upon changing the primary ion's activity. Then, the interfering ion would be added to an identical reference solution until the same potential change is obtained. The selectivity coefficient, $K_{A,B}^{Pot}$, is determined with the following equation:

$$K_{A,B}^{Pot} = \frac{\Delta A}{a_B} \text{ where } \Delta A = a'_A - a_A$$

in which a_A is the initial primary ion activity and a'_A is the activity of A in the presence of the interfering ion, a_B . It should be noted that the concentration of Fe^{3+} ion used as a primary ion in the present study was 1.0×10^{-4} M. The obtained selectivity coefficients are summarized in Table 2. The data given in Table 2 indicate that the resulting selectivity coefficient of the proposed electrode for Fe^{3+} was much larger in comparison to other cations, such as Co^{2+} , Cu^{2+} , and especially Fe^{2+} , probably due to a specific interaction between the ionophore and Fe^{3+} ions, and its low tendency toward common inorganic cations. Therefore, it may be stated that the proposed membrane electrode showed high selectivity toward Fe^{3+} and foreign cations did not cause any interference with the functioning of the proposed Fe^{3+} ion-selective sensor, even at high concentration levels (Figure 2).

Table 2. Selectivity coefficients of various interfering ions for Fe^{3+} ion selectivity.

Interfering ion	$-\log k_{Fe/I}^{MPM}$
Cd^{2+}	2.35 ± 0.06
Zn^{2+}	1.95 ± 0.11
Mn^{2+}	No response
Ca^{2+}	3.52 ± 0.05
Cu^{2+}	2.12 ± 0.04
Co^{2+}	3.11 ± 0.03
Ba^{2+}	No response
Ag^+	1.77 ± 0.05
Fe^{+2}	2.18 ± 0.15

Under optimal conditions the EMF response of the sensor in response to varying the activity of the Fe^{3+} ions indicated a linear dynamic range of 5.2×10^{-6} - 1.0×10^{-2} M. The slopes of the calibration curve were 19.5 ± 1.5 mV per decade of the Fe^{3+} concentration. The limit of detection, as determined by the intersection of the 2 extrapolated segments of the calibration graphs, was 1.2×10^{-6} M.

In Table 3 the analytical performance of the membrane electrode based on 6-KMPT (present work) are compared with the corresponding values previously reported for PVC membrane Fe(III) ion-selective electrodes based on $[\text{Fe}(\text{citrate})_2]^{3-}$ -tricaprylylmethylammonium,⁸ iron-cyclam,⁹ iron-HPDTP,¹⁰ p-chloroaniline-3-formylsalicylic acid,¹¹ chalcogenid glass membrane,¹² 1,7-dithia 12-crown-4,¹³ 5,10,15,20-tetrakisPFP-21-H-23-H-porphyrine,¹⁴ and tetrachloroferrat(III) TCM-ammonium chloride.¹⁵ As can be seen, a wider linear range and a full Nernstian response slope are absolute advantages over the previously reported PVC Fe^{3+} selective membrane electrodes. Moreover, the ionophore used in the present study showed selectivity that was somewhat similar in most cases, or even superior in some cases, to that of the previously reported sensors.

Analytical Applications

Potentiometric Titration of Fe³⁺ Ions

The practical utility of the proposed Fe³⁺ membrane sensor was initially tested by using it as an indicator electrode for the titration of 10 mL of a 1.0×10^{-3} M Fe³⁺ ion solution with 1.0×10^{-2} M EDTA. The resulting titration curve is shown in Figure 4. A very good inflection point that corresponded to Fe³⁺-EDTA stoichiometry was observed, which indicated the suitability of the proposed sensor for the potentiometric determination of Fe³⁺ ions.

Table 3. Comparison of the analytical characteristics of Fe³⁺ ion-selective electrodes.

Linear response range (M)	Slope (mV per decade)	Ionophore	Reference
$5.2 \times 10^{-6} - 1.0 \times 10^{-2}$	19.5	KMPT	This work
$1.0 \times 10^{-3} - 1.0 \times 10^{-1}$	19.3	[Fe(citrate) ₂] ³⁻ -tricaprylylmethylammonium	8
$1.0 \times 10^{-6} - 1.0 \times 10^{-2}$	60	Iron-Cyclam	9
$3.5 \times 10^{-6} - 4.0 \times 10^{-2}$	28.5	Iron-HPDTP	10
$5.0 \times 10^{-5} - 1.0 \times 10^{-1}$	20	p-chloroaniline-3-formylsalicylic acid	11
$1.0 \times 10^{-7} - 1.0 \times 10^{-2}$	60	chalcogenid glass membrane	12
$1 \times 10^{-5} - 1 \times 10^{-3}$	56 (monovalent)	1,7-dithia 12-crown-4	13
$1 \times 10^{-6} - 1 \times 10^{-4}$	25	5,10,15,20-tetrakisPFP-21-H-23-H-porphyrine	14
$1 \times 10^{-4} - 1 \times 10^{-1}$	near Nernst slope	tetrachloroferrat (III)TCM-ammonium chloride	15

Determination of Iron in Pharmaceuticals

Due to the vital importance of the rapid assay of pharmaceutical products, in recent years potentiometric measurement using ion-selective electrodes has found widespread use in pharmaceutical and clinical analysis, and in the study of drug interactions with other chemicals and biochemicals. The proposed Fe³⁺ ion-selective electrode was also successfully used as an indicator electrode for the determination of the presence of iron in ferrous sulfate tablets via potentiometric titration and the results were in good agreement with data obtained by atomic absorption spectrophotometry (AAS). Mean values of 5 recoveries obtained with the proposed and AAS methods were $99.75\% \pm 0.41$ and $99.96\% \pm 0.83$, respectively. T-test results (Table 4) show that there were no significant differences between the 2 methods in terms of mean recovery ($P > 0.05$).²⁹

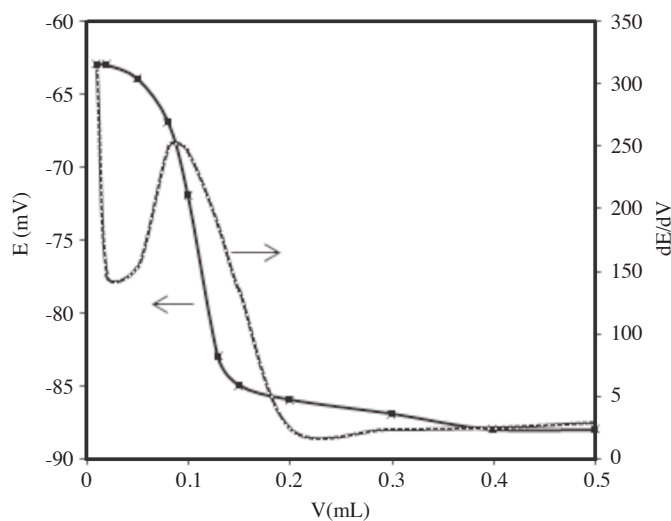


Figure 4. Potentiometric titration curve of Fe^{3+} ions (1.0×10^{-4} M, 10 mL) with EDTA (1.0×10^{-2} M).

Table 4. Statistical comparison of the results of the analysis of ferrous sulfate tablets (labeled 25 mg) obtained with the proposed sensor and AAS methods.

Parameter	Proposed method	AAS method
Fe (mg)	25.12	25.25
Mean recovery (%) ^a	99.75	99.96
SD	0.41	0.83
F-ratio ^b	4.09	
t-test	0.78	

^a Average of 5 measurements.

^b Theoretical values of F and t are 6.39 and 2.31, respectively, at the 95% confidence level.

Conclusions

The present study showed that 6-KMPT could be used as a carrier in the preparation of a PVC membrane that was plasticized with DOP for the highly selective monitoring of Fe^{3+} ions in the presence of other common cations. Analytical characteristics of the proposed Fe^{3+} ion-selective electrode were compared to previously reported Fe(III) ion-selective electrodes. The wide dynamic concentration range, low detection limit, fast response time, and high selectivity indicate that this sensor would be suitable for measuring the concentration of Fe^{3+} ions in various samples, without the need for pre-treatment steps. Finally, it was successfully used for determining the presence of iron in pharmaceutical preparations.

Acknowledgments

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