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PVC membrane sensor for potentiometric determination of iron (II) in some pharmaceutical formulations based on a new neutral ionophore

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A novel poly (vinyl chloride) PVC membrane sensor for Fe^{2+} ions is described. The sensor is based on the use of newly synthesized chiral 2,6-bis-(carboxamide methyl ester)pyridine derivative as neutral ionophore in plasticized PVC membrane. The sensor display a fast, stable and near-Nernstian response over a relative wide ferrous concentration range (1×10^{-3} to 6×10^{-6} M), with cationic slope of 31.5 ± 0.5, mV per concentration decade over a pH range of 5.0–9.0. The direct determination of 0.25–56.0 μg/ml of ferrous in aqueous solution shows an average recovery of 98.5% and a mean relative standard deviation of 1.5% at 20.0 μg/ml. The sensor displays long life-span, long-term stability, high reproducibility, and short response time. Selectivity coefficients for Fe(II) relative to a number of interfering substances were investigated. The sensor shows high significantly for Fe^{2+} over $Fe,^{3+}$ $Cu,^{2+}$ $Zn,^{2+}$ $Cd,^{2+}$ $Hg,^{2+}$ $Pb,^{2+}$ $Ni,^{2+}$ $Co,^{2+}$ $Mn,^{2+}$ $Al,^{3+}$ alkaline earth and alkali metal ions. The sensor is successfully applied for measurement of ferrous in drug formulations. The results obtained for the determination of ferrous using the proposed sensor are comparable favourably with those obtained using the spectrophotometric method.

Keywords: ferrous; chiral 2,6-bis-(carboxamide methyl ester)pyridine derivative; Ionophore; multivitamins

Introduction

Iron is one of the vitally important metals. Iron ions are found in active sites in hemoglobin, myoglobin, cytochromes, catalase and many other proteins that realize oxygen and electron transport and enzyme functions. Therefore, a disorder of iron metabolism is among the most common diseases of humans.[1] For instance, iron deficiency causes anaemia and other pathological changes in the body, and anaemia remains an important public health problem.[2] To prevent and treat iron deficiency, some iron-containing pharmaceutical products are used. These medicines may be oral iron containing vitamins and dietary supplements[3–6] or injectable iron containing pharmaceuticals.[7–9]

Various methods cited in literature for its determinations involve flame atomic absorption spectrophotometry,[10] spectrophotometry,[11–14] fluorimetry,[15,16] chemiluminescence,[17,18] differential pulse voltammetry,[19] and capillary electrophoresis.[20–22]

Recent years have seen an upsurge of interest in the application of sensors in the field of medicinal analysis.[23–26] This can be explained by the good analytical performances in terms of selectivity, accuracy, low detection limit, wide concentration range, and relatively limited financial investment. To our knowledge till now, no potentiometric membrane sensor for ferrous has been published.

The only reported PVC membrane senor was based on N-phenylaza-15-crown-5 as neutral carrier in PVC matrix.[27] The proposed sensor showed a fairly good discriminating ability towards Fe^{2+} ion in comparison with some hard and soft metal ions.

On the other hand, carboxamides and peptides are very effective and often act as specific ligands for a variety of metal ions due to the presence of potential donor atoms in their backbone. Acyclic and cyclic peptides exist in a variety of conformations and their binding to metal ions involving participation of either the carbonyl oxygen or the amide nitrogen. Simple synthesis of different carboximide and peptides derivatives renders these ligands a highly attractive class of electroactive materials for developing potentiometric metal ion sensors.[28–31]

In the present work, a novel synthesized ferrous polymeric membrane sensor incorporating novel a chiral 2, 6-bis (carboxamide methyl ester) pyridine derivative has been investigated. A sensor based on new ionophore offers the advantages of lower detection limit, fast response time, long-term stability, near-Nernstian slope over a wide range of concentration, and remarkable selectivity for Fe^{2+} ion over most common cations.

Experimental

Synthesis of the ionophore

Method A (mixed anhydride method)

To a stirred cold mixture (−15 °C) of 2,6-pyridine dicarboxylic acid **(1)** (0.167 g, 1 mmol) in cold dry tetrahydrofuran (100 ml) and ethyl chloroformate (0.216 g, 2 mmol), triethyl amine (0.202 g,

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2 mmol) was added. After 10 min, D-alanyl methyl ester (0.206 g, 2 mmol) [prepared by adding triethylamine (0.202 g, 2 mmol) to D-alanyl methyl ester hydrochloride (0.280 g, 2 mmol) in 25 ml dry tetrahydrofuran at (−10 °C) with stirring for 10 min was added. The reaction mixture was stirred at the same temperature for 3 h and for 12 h at room temperature. The formed triethylamine hydrochloride was filtered off, and the solvent was evaporated under reduced pressure. The obtained residue was dissolved in dichloromethane (150 ml), washed with water, 1N hydrochloric acid, 1N sodium bicarbonate, and finally with water and dried over anhydrous calcium chloride. The solvent was evaporated under reduced pressure to dryness and the obtained solid was crystallized from methanol/ether to give the corresponding 2,6-bis- ester derivative **3**, in 74% yield.

Method B (acid chloride method)

To a solution of D-alanyl methyl ester (0.206 g, 2 mmol), obtained by the addition of triethylamine (0.202 g, 2 mmol) to the D-alanyl methyl ester hydrochloride (0.280 g, 2 mmol) in dry dichloromethane (50 ml) at (−15 °C) and stirring for 15 min, 2,6-pyridinedicarbonyl dichloride (0.204 g, 1 mmol) in dry dichloromethane (15 ml) was added at the same condition. Triethylamine (0.202 g, 2 mmol) was drop wisely added to the reaction mixture during stirring in order to keep the reaction mixture slightly basic (pH ∼8). Stirring was maintained for 3 h at (−15 °C) and 12 h at room temperature. The reaction mixture was then washed with water, 1N hydrochloric acid, 1N sodium bicarbonate, and finally with water and dried over anhydrous calcium chloride. The solvent was evaporated under reduced pressure to dryness and the obtained solid was crystallized from ethanol/ether to give **3**. Yield (80%); M. p. 148 °C; IR (KBr): ν = 3284 (NH), 1742 (C═O, ester), 1680 (C═O, amide) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.56 (d, 6H, 2 CH_3), 3.80 (s, 6H, 2OCH_3), 4.70–4.81 (m, 2H, 2 CH), 8.05 (s, 2H, 2 NH exchangeable with D_2O), 8.29–8.37 (m, 3H, Pyrid-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.20, 48.31, 52.60, 125.19, 138.93, 148.35, 162.89, 173.28; MS (EI, 70 eV): *m/z* (%) = 237 (M^+, 4), 306 (24), 275 (100), 204 (36), 133 (18), 105 (65), 77 (86); $C_{15}H_{19}N_3O_6$ (337.33): Calculated. C, 53.41; H, 5.68; N, 12.46. Found: C, 53.36; H, 5.64; N, 12.41.

Apparatus

All potentiometric measurements were made at 25 ± 1 °C unless otherwise stated using an Orion pH/mV meter (model 330) using ferrous membrane sensor in conjunction with an Orion double junction Ag/AgCl reference electrode (model 90-02) containing 10% (*w/v*) potassium chloride in the outer compartment. Adjustment of pH was made with a combined Ross glass pH electrode (Orion 81-02) for all pH measurements.

Reagents and materials

All chemicals used were of analytical reagent grade unless otherwise stated and doubly distilled water was used throughout. Polyvinyl chloride powder (PVC) high molecular weight, dibutyl sebacate (DBS), dioctyl phthalate (DOP), o-nitrophenyl octylether (NPOE), sodium tetraphenyl borate (Na TPB), and tetrahydrofurane (THF) of purity >99%, were obtained from Aldrich Chemical Company Germany, Steinheim. Ferrous (II) *sulfate* was obtained from Sigma-Aldrich Company, German, Steinheim. The stock solution of 1×10^{-2} M ferrous was prepared by dissolving the appropriate amount of ferrous (II) sulphate in 100 ml water and the pH of solution was adjusted to pH 6. The standard ferrous (II) solution was prepared (1×10^{-3}–1×10^{-6}) by diluting the appreciate amount in 0.03 M KNO_3. Multivitamin, Centrum, USA New York; Multivitamin with iron syrup, HAPPY DEEF with iron, KSA Al Qasim; Ferrous Glyconate tablets, KSA, Riyadh.

Preparation of the Fe(II)-PVC membrane sensor

PVC-based ferrous membrane sensor was prepared using 10 mg of ionosphere, 350 mg of DOP or DBS or NPOE plasticizer and 190 mg PVC dissolved in ∼5 ml THF in glass Petri dishes (5 cm diameter). After the constituents being well mixed, the solvent was allowed to evaporate overnight while the sensing membranes were formed. The PVC master membranes were sectioned with a cork borer (10 mm diameter) and glued to a polyethylene tube (3 cm length, 8 mm I.D.) using THF.[29,32] Laboratory-made electrode bodies were used, which consisted of a glass tube, to which the polyethylene tube was attached at one end and filled with internal reference solution (equal volumes of 1×10^{-2} M aqueous solution of ferrous (II) and KCl). Ag/AgCl internal reference electrode (1.0 mm diameters) was used. The indicator electrode was conditioned by soaking in a 1×10^{-2} M aqueous ferrous (II) solution for 1 h and stored in the same solution when not in use.

Procedure

The ferrous PVC membrane sensor was calibrated by immersion in conjunction with the reference electrode in a 50-ml beaker containing ferrous solutions. The sample solutions were ferrous over the concentration range 1×10^{-3} to 1×10^{-6} M at pH 6.0 and at constant ionic strength of 0.03 M KNO_3. Calibration graphs were then constructed by plotting the recorded potentials as a function of $-\log [Fe^{2+}]$. The resulting graphs were used for subsequent determination of unknown ferrous concentration.

Determination of ferrous in multivitamin dosage forms

Ten tablets of ferrous glyconate or multivitamin (300 or 18 mg of ferrous) were accurately weighed and crushed and mixed in a mortar. An appropriate amount (300 mg or 18 mg of ferrous powder, from each) was weighed, transferred to a 100-ml beaker and dissolved in distilled water, sonicated for about 15 min and completed to the mark with water. A 5.0-ml aliquot of this solution was transferred to 50-ml standard flask and the pH of solution was adjusted at pH 6.0, and completed to the mark with 0.03 KNO_3.

Ten ml of ferrous syrup was transferred into 50-ml measuring flask, diluted with water, shaking well and sonicated for 10 min then completed to the mark with water. An appropriate volume was transfer into 25-ml measuring flask and completed to the mark with 0.03 M KNO_3.

The potential of the above solutions were measured using ferrous sensor in conjunction with an Orion Ag/AgCl double junction reference electrode. The potential of the stirred solution was recorded after the signal stabilization (±1 mV/min) and the concentration was calculated from the previous calibration graph under identical experimental conditions from standard solutions of ferrous.

Results and discussion

A chiral 2, 6-bis (carboxamide methyl ester) pyridine derivative (Figure 1) was prepared and examined as a novel natural carrier for ferrous ion in PVC matrix membrane sensor. Three membrane

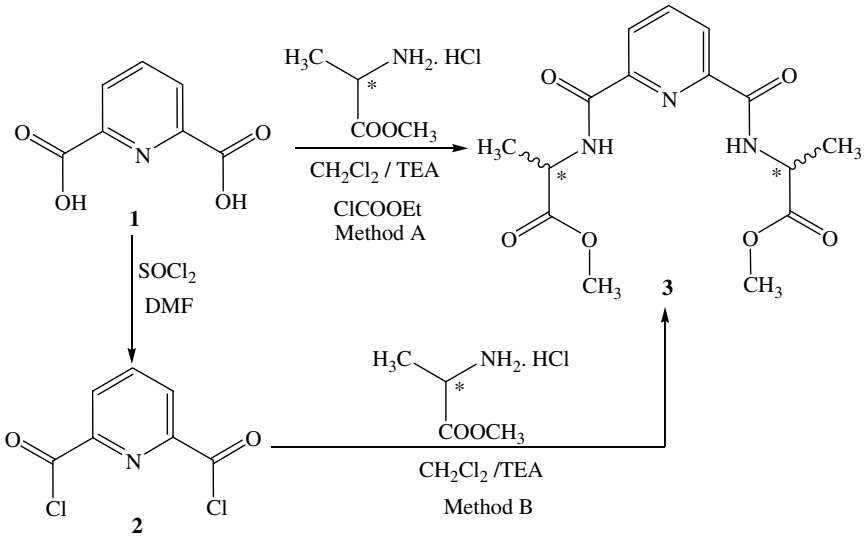

Figure 1. The synthetic route of ionophore **(3)**.

Table 1. Effect of membrane components on the performance characteristics of ferrous sensor based on 2, 6-bis-(carboxamide methyl ester) pyridine derivative

MEMBRANE COMPONENT	SLOPE	LINEARITY
PVC + DBS	–	–
PVC + DBS + ionophor	31.5 ± 0.5	1×10^{-3}–6×10^{-6}
PVC + DBS + ionophore + NaTPB	22.5 ± 0.5	1×10^{-3}–8×10^{-6}

sensors for the proposed ionophore were prepared and evaluated during three months according to IUPAC recommendation[33] (Tables 1–3). Potentiometric characteristics of sensor incorporating ionophore revealed strong response for ferrous ions over other metal ions, anions and other excipients (Table 3). Although neutral carrier-based ISE membranes may work properly even when they contain only very small amount of ionic sites (e.g. as impurities), the addition of a salt of lipophilic ion is advisable and beneficial for various other reasons as well. In fact, it has been demonstrated that the presence of lipophilic negatively charged additives improves the potentiometric behaviour of certain cation selective electrodes by reducing the ohmic resistance and improving the response behaviour and selectivity[34] and in some cases, by catalyzing the exchange kinetics at the sample membrane interface.[35] From the data presented in Table 1, it is seen that the addition of NaTPB decreases the sensitivity of the electrode response considerably. So in this case, we use the proposed ionophore without the addition of NaTPB.

Sensors characteristics

The potentiometric response characteristics of the ferrous sensor based on the use of synthesized ionophore as an electroactive materials and DBS or DOP or NPOE as a plasticizer in a PVC matrix were evaluated according to IUPAC recommendations.[33] Results in Table 2 show the characteristics performance of the PVC membrane sensor. The least squares equation obtained from the calibration data as follows:

$$E(\text{mV}) = S \log [Fe^{2+}] + \text{Intercept}, \quad (1)$$

Table 2. Response characteristics of ferrous-PVC matrix membrane sensor

Parameter	Ferrous-PVC sensor
Slope, (mV/decade)	31.5 ± 0.5
Intercept, mV	114.5 ± 0.5
Correlation Coefficient, (r)	0.998
Lower limit of quantification (LLQ), M	6×10^{-6}
Lower limit of detection (LLD), M	4×10^{-6}
Response time for 1×10^{-3} M solution, sec	30
Working pH range	5.0–9.0
Life time of the electrode, day	**60**

Table 3. Potentiometric selectivity coefficients of some interfering ions, using ferrous membrane sensor

Interferent, J	$K^{pot}_{Fe(II),B}$ MPM	Interferent, J	$K^{pot}_{Fe(II),B}$ MPM
Na^+	6.0×10^{-4}	Magnesium Stearate	1.0×10^{-3}
K^+	6.0×10^{-4}	Phosphate	6.0×10^{-4}
Ag^+	6.0×10^{-4}	Acetate	6.0×10^{-4}
NH_4^+	6.0×10^{-4}	Citrate	6.0×10^{-4}
Ca^{2+}	6.0×10^{-4}	Nitrate	6.0×10^{-4}
Zn^{2+}	2.0×10^{-2}	Nitrite	6.0×10^{-4}
pb^{2+}	3.0×10^{-2}	chromate	6.0×10^{-4}
Cu^{2+}	1.8×10^{-2}	Perchlorate	6.0×10^{-4}
Cd^{2+}	1.0×10^{-3}	Thiocyanate	6.0×10^{-4}
Hg^{2+}	1.0×10^{-3}	Sulphate	6.0×10^{-4}
Zn^{2+}	6.0×10^{-4}	L-Tryptophane	1.0×10^{-3}
Mn^{2+}	6.0×10^{-4}	DL-Alanine	1.0×10^{-3}
Mg^{2+}	6.0×10^{-4}	Glycine	1.0×10^{-3}
Co^{2+}	6.0×10^{-4}	Glucose	1.0×10^{-3}
Ni^{2+}	6.0×10^{-4}	Lactose monohydrate	1.0×10^{-3}
Al^{3+}	6.0×10^{-4}	Starch	1.0×10^{-3}
Fe^{3+}	6.0×10^{-4}	Microcrystalline cellulose	1.0×10^{-3}

where *E*, is the potential of the electrode, *S* equal slope of the electrodes (31.5±0.5) and intercept (114.5±0.5) mV, respectively).

Effect of plasticizer type on the characteristic performance of the sensors

Ferrous ion-selective membrane sensors with the new ionophore were investigated in different plasticizer order to compare their performance. Three used PVC membranes were investigated as possible counter ions for the preparation of the electroactive material of ferrous. The obtained ionophore combined with three plasticizers namely DOP or DBS or NPOE to give different combinations were tested. It is well known that the construction of PVC-based ISEs requires the use of a plasticizer which acts as a fluidizer allowing homogenous dissolution and diffusion mobility of the ionophores inside the membrane. PVC membrane sensor of ferrous (II) with different plasticizer, namely DBS, DOP or NPOE, was found to be the optimum available mediator for ferrous membrane sensor. It plasticizes the membrane, dissolves the ionophore and adjusts both the membrane permittivity and ion-exchanger sites mobility to give the highest possible selectivity and sensitivity. It can be seen that membranes incorporating DBS ($\varepsilon = 4$) plasticizer gave more favourable results than those containing DOP ($\varepsilon = 7$) or NOPE ($\varepsilon = 24$). In general, membranes incorporating DBS plasticizer give more favourable linear range, slope, and low detection limit than those containing DOP and NPOE plasticizer. The calibration graphs, slopes, and lower limits of detection were obtained by using DBS, DOP, and NPOE as follows: 1×10^{-3}–6×10^{-6}, 1×10^{-3}–1×10^{-5}, 1×10^{-3}–1×10^{-5} M; 31.5±0.5, 24.0±0.7, 26.0±0.5 and 8×10^{-6}, 8×10^{-6}, 4×10^{-6} M for DBS, DOP, and NPOE, respectively. Low dielectric constant solvent mediators have been previously recommended for some divalent metal cation membrane sensors.[36,37] In this study, all subsequent measurements were made with membranes plasticized with DBS.

Effect of pH and the response time

The electrode response for different ferrous concentration was tested at different pH values, the pH being adjusted using hydrochloric acid or sodium hydroxide. The Fe(II)-PVC membrane electrode dipped into ferrous solution of 1×10^{-3} M was plotted against the pH of solution (Figure 2). The figure shows that the potential of the proposed sensor was constant over the pH range of 5–9. The observed change in potential response at lower pH (<5.0) may be due to the protonation of the ionophore, which results in an increasing concentration of hydrogen ion in solution. Therefore, in all incoming measurement should be in the pH range of 5–9.

The average response time is defined[33] as the time required for the electrode to reach a stable potential within ±1 mV of the final equilibrium value, after successive immersion of the electrode in different ferrous solutions each having a ten-fold difference in concentration or after rapid ten-fold increase in concentration by addition of ferrous. This time was found to be 25s for concentration of $\geq 1 \times 10^{-4}$ M and 30 s for concentration $\leq 1 \times 10^{-5}$ M. Day-to-day reproducibility of the sensor is about ±0.5 mV for the same solution and the useful lifetime of the sensor is two months, during which the potential slope is reproducible to within ±1 mV/decade. Also after more than one month, a new section from the master membrane was found to function properly.

Effect of diverse ions

The influences of different organic and inorganic ions on the response of ferrous sensor were investigated. The selectivity coefficients $K_{A,B}^{pot}$ were evaluated according to IUPAC guidelines using the match potential method (MPM)[38,39] in 0.03 M KNO_3 solution. Match potential method, the potentiometric selectivity coefficient is defined as the activity ratio of primary ion and interfering ions that give the same potential under identical conditions. At first, a known activity (a'_A) of the primary ion solution is added into a reference solution that contains a fixed activity (a_A) of primary ions, and the corresponding potential change (ΔE) is recorded. Next, a solution of an interfering ion (a_B) is added to the reference solution until the same potential change (ΔE) is recorded. The change in potential produced at the constant background of the primary ion must be the same in both cases. The selectivity coefficient is calculated from Equation 2:

$$K_{A,B}^{pot} = (a'_A - a_A)/a_B \quad (2)$$

On the other hand, the ferrous-PVC membrane sensor is free from the interferences of large number of anions, for example, perchlorate, thiocyanate, cyanide, sulfate, sulfite, nitrate, nitrite, chromate, chloride, bromide, iodide, phosphate, acetate, bicarbonate. The results given in Table 3 reveal reasonable selectivity for ferrous ion in presence of many related substances.

Validity of the proposed method

Limit of quantification and limit of detection

Each of different concentration of standard solution was tested five times. The potentials obtained for five analyses were averaged at each concentration. The average potential was plotted versus concentration. The relation between potential and concentration is logarithmic (Equation 1), $X = S \log [Fe(II)] + Y$, where *X* is equal the potential, S slope, Y is the intercept and r is the correlation coefficient. The sensor display a linear response over the concentration range of 1×10^{-3}–6×10^{-6} M ferrous (II) with a Nernstian cationic slope of 31.5 ± 0.5 mV/concentration decade.

The lower limit of detection (LOD) was estimated and confirmed by two criteria: the first one is 3 sigma and the second one is relative standard deviation (RSD %) which is 2.0% or less for five replicate. Also the LOD limit was defined as the concentration of ferrous corresponding to the intersection of the extrapolated linear segment of the calibration graph, which is 4×10^{-6} M. The lower limit of quantification (LOQ) was defined as 10 sigma.

Precision and accuracy of the method

The precision and accuracy of the method were investigated by inter-day (repeatability) analysis of ferrous, five replicate at the LOQ range. The precision and accuracy of the method are expressed as RSD and % of deviation of the measured concentration. Also reproducibility (day to day or intra-day) was investigated. The results obtained (Table 4) are within the acceptance range of less than 1.8% (precision) and 2.0 (accuracy).

Ruggedness

The ruggedness of the potentiometric method was evaluated by carrying out the analysis using two different analysts (operators) and different instruments on different days. The RSD of less than

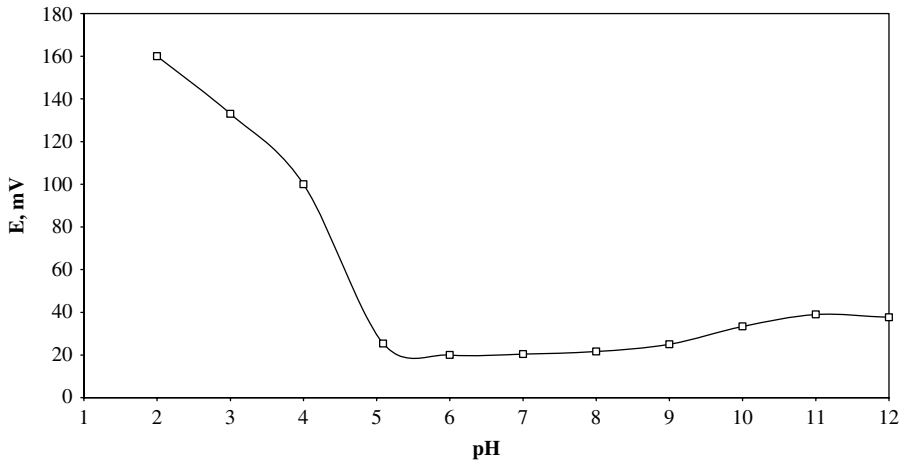

Figure 2. Effect of pH on the response of Fe (II)-PVC membrane sensor using 1×10^{-3} M of Fe(II).

Table 4. Day to day reproducibility of the proposed ferrous membrane sensor

Parameter	Ferrous (20 µg/ml)* Within-day ferrous-PVC sensor	Ferrous (20 µg/ml)* Within-days ferrous-PVC sensor
R, % ± RSD	98.5 ± 1.8	98.5 ± 2.0
R.S.D, %	1.8	2.0
Slope	30.0 ± 0.5	30.5 ± 0.6
Correlation coefficient	0.998	0.998

* Average of 5 measurements ± RSD.
* R %, recovery percentage; RSD relative standard deviation.

Table 5. Direct determinations of ferrous using PVC membrane sensor

Added (µg/ml)	Recovery, % ± RSD Ferrous PVC sensor
0.25	97.5 ± 1.8
1.0	97.5 ± 1.8
2.5	98.0 ± 1.8
5.0	98.2 ± 1.7
10.0	98.5 ± 1.7
20.0	98.5 ± 1.7
50.0	99.0 ± 1.7

* Average of 5 measurements ± RSD.

2.0% were observed for repetitive measurements in three different daytime periods using two different instruments and operators. The results indicate that the method is capable of producing results with high precision.

Robustness

The robustness of the method is demonstrated by the versatility of the experimental factors that affect the potential response. Preliminary inspection of the results (pH range from 5 to 9, the electrode potential response after 25 s or more and lifetime of electrode from 1 day to 60 days give a good response with RSD $\leq$2%) under these various conditions suggested that the method is fairly robust, but the pH of the measuring solution should be in the range 5.0–9.0.

Determination of ferrous

The applicability of the ferrous membrane sensor for determination of ferrous was first checked by the studying the recovery of an accurate amount of pure ferrous in solutions.

The direct determinations of iron (II) were carried out using the developed membrane sensor. The analysis of 0.2–56.0 µg/ml ferrous solutions (in five replicates) by direct potentiometry gave an average recovery of 98.5 ± 1.7 for use the proposed sensor, at 20 µg/ml (results are shown in Table 5).

The applicability of ferrous-PVC membrane to the determination of ferrous ion in the dosage forms was first checked by studying the recovery of an accurate amount of ferrous in reconsitutent powder samples. The recovery obtained for five measurements was found to be 98.5% with a relative standard deviation of 1.8% for ferrous sensor. Results obtained for the analysis of ferrous in each formulation by direct measurements using the proposed sensor and the validated spectrophotometric method [14] are given in Table 6. These data suggest the proposed method can be carried out on real products with equal confidence and accuracy.

Comparison between the experimental means for the two methods was carried out using the null hypothesis of $|t|_2$ for P = 0.05 and n = 5. It was found that $|t|_2$ = 1.2, 1.4 and 1.7 which is less than the tabulated value ($|t|_2$ = 3.36).[40] No significant difference was found between the two methods, which indicates that the proposed method is as accurate as the spectrophotometric method. Comparison between the precision of the proposed method with the spectrophotometric method to estimate the random errors of the two sets of data (Table 6) was also carried out using the two-tailed F-test.[40] From this table, it is clear that all the experimental $F_{4,4}$ values are 1.4, 1.5, and 1.7. These values are obviously less than the tabulated value of $F_{4,4}$ for P = 0.05 and n = 5 (6.38).[40] This proves that the results obtained by the two methods are not subject to random errors.

Conclusion

Experimental comparison of the synthesized ionophore as electroactive material and different plasticizer in potentiometric

Table 6. Determination of ferrous in some pharmaceutical formulation using the proposed membrane sensor

Drug, name	Ferrous (nominal,value)	Proposed method* R, % ± RSD	Spectrophotometry R, % ± RSD	$\|t\|_2$	F-test
Ferrous Glyconate	300 mg	99.5 ± 1.6	99.4 ± 1.6	1.2	1.4
Multivitamin, Centrum	18 mg	98.5 ± 1.7	98.5 ± 1.8	1.4	1.5
Multivitamin, Syrup	5 mg	98.0 ± 1.8	99.0 ± 2.0	1.5	1.7

* Average of 5 determinations.

membrane sensor revealed that in most cases, the ferrous membrane sensor displayed good analytical performance characteristics. The sensitivity, linear range, and slope are independent over the pH rang 5.0–9.0. The application of the proposed sensor to the determination of ferrous in its pure solutions, ferrous tablets, and multivitamin formulations are characterized by a high degree of precision and accuracy compared to the validated spectrophotometric method.

References

[1] P. T. Lieu, M. Heiskala, P. A. Peterson, Y. Yang. The roles of iron in health and disease. *Mol. Aspects Med.* **2001**, *22*, 1.
[2] N. C. Andrews. Iron metabolism: iron deficiency and iron overload. *Annu. Rev. Genomics Hum. Genet.* **2000**, *1*, 75.
[3] S. Zlotkin, P. Arthur, K. Y. Antwi, G. Yeung. Randomized, controlled trial of single versus 3-times-daily ferrous sulfate drops for treatment of anemia. *Pediatrics.* **2001**, *108*, 613.
[4] S. Zlotkin, P. Arthur, K. Y. Antwi, G. Yeung. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods. *Am. J. Clin. Nutr.* **2001**, *74*, 791.
[5] O. Pineda, H. D. Ashmead. Effectiveness of treatment of iron-deficiency anemia in infants and young children with ferrous bis-glycinate chelate. *Nutrition* **2001**, *17*, 381.
[6] M. I. Oshtrakh, O. B. Milder, V. A. Semionkin. Determination of the iron state in ferrous iron containing vitamins and dietary supplements: application of Mössbauer spectroscopy. *J. Pharm. Biomed. Anal.* **2006**, *40*, 1281.
[7] S. C. Szarfarc, L. M. de Cassana, E. Fujimori, E. M. Guerra-Shinohara, I. M. de Oliveira. Relative effectiveness of iron bis-glycinate chelate (ferrochel) and ferrous sulfate in the control of iron deficiency in pregnant women. *Arch. Latinoam. Nutr.* **2001**, *51*, S42.
[8] J. C. Barton, E. H. Barton, L. F. Bertoli, C. H. Gothard, J. S. Sherrer. Intravenous iron dextran therapy in patients with iron deficiency and normal renal function who failed to respond to or did not tolerate oral iron supplementation. *Am. J. Med.* **2000**, *109*, 27.
[9] M. Manuel, G. R. Susana, A. G. José. Intravenous iron in inflammatory bowel disease. *World J. Gastroentero.* **2009**, *15*, 4666.
[10] H. Bag, A. Rehber, A. Tunceli, M. Lale. Determination of iron(II) and iron(III) in water by flame atomic absorption spectrophotometry after their separation with Aspergillus niger immobilized on sepiolite. *Anal. Sci.* **2001**, *17*, 901.
[11] A. M. Ali, M. Yoshihito, S. Kiyoshi. Solvent sublation and spectrometric determination of iron(II) and total iron using 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine and tetrabutylammonium bromide. *Anal. Sci.* **2006**, *22*, 1169.
[12] A. A. Ensafi, C. M. Arab, M. H. Rahimi. Sequential determination of iron (II) and iron(III) in pharmaceutical by flow-injection analysis with spectrophotometric detection. *Anal. Sci.* **2004**, *20*, 645.
[13] L. V. Mulaudzi, J. F. van Staden, R. I. Stefan. On-line determination of iron(II) and iron(III) using a spectrophotometric sequential injection system. *Anal. Chim. Acta* **2002**, *467*, 35.
[14] Z. Marczenko. *Spectrophotometric Determination of Elements*, Ellis Hardwood Ltd: Sussex, **1976**, pp. 305.
[15] C. Jin-Long, Z. Shu-Juan, W. Yu-Qing, F. Fang, L. Ling, Z. Chang-Qing. High selective determination of iron(II) by its enhancement effect on the fluorescence of pyrene-tetramethylpiperidinyl (TEMPO) as a spin fluorescence probe. *Spectrochim. Acta A* **2006**, *63A*, 438.
[16] Z. Guohui, Z. Zhancai, Q. Lanfang. A fluorometric method for the determination of iron(II) with fluorescein isothiocyanate and iodine. *Anal. Sci.* **2002**, *18*, 1059.
[17] M. Yaqoob, S. J. Ussher, A. Nabi, E. P. Achterberg, P. J. Worsfold. Direct determination of iron(II) in acidified seawater using a matrix matched flow injection manifold with chemiluminescence detection. *J. Flow Injection. Anal.* **2003**, *20*, 183.
[18] H. Shizuko, Y. Hiroki, A. Masato. Determination of iron(II) and total iron in environmental water samples by flow injection analysis with column preconcentration of chelating resin functionalized with N-hydroxyethylethylenediamine ligands and chemiluminescence detection. *Talanta* **1999**, *49*, 1059.
[19] K. K. Wone, K. H. Cheol, K. Sung-Hyun, P. B. Ho, K. Y. Hee, K. K. Nam, K. Y. Chun. Differential pulse voltammetric determination of iron(II) ion with a Nafion-Ethylenediamine Modified Glassy Carbon Electrode. *J. Korean Chem. Soc.* **2003**, *47*, 115.
[20] T. Yoshitaka, I. Shukuro. Simultaneous determination of iron(II) and iron (III) by micellar electrokinetic chromatography using an off-line selective complexing reaction, *Anal. Sci.* **2003**, *19*, 1207.
[21] D. Johan, K. Stefan. Determination of iron(II) in natural waters by capillary zone electrophoresis using on-capillary complexation with 2,4,6-tri(2′-pyridyl)-1,3,5-triazine. *J. Chromatogr. A* **1999**, *848*, 491.
[22] S. Pozdniakova, A. Padarauskas, G. Schwedt. Simultaneous determination of iron (II) and iron (III) in water by capillary electrophoresis. *Anal. Chim. Acta* **1997**, *351*, 41.
[23] S. Riahi, M. F. Mousavi, S. Z. Bathaie, M. Shamsipur. A novel potentiometric sensor for selective determination of theophylline: Theoretical and practical investigations. *Anal. Chim. Acta* **2005**, *548*, 192.
[24] M. Shamsipur, F. Jalali, S. Ershad. Preparation of a diclofenac potentiometric sensor and its application to pharmaceutical analysis and to drug recovery from biological fluids. *J. Pharm. Biomed. Anal.* **2005**, *37*, 943.
[25] J. Yuan, S. Yao. A sulfa-drug sensitive sensor based on ion-pair complex modified PQC resonator. *Talanta* **2002**, *58*, 641.
[26] G. A. E. Mostafa, A. Al-Majed. Characteristics of new composite- and classical potentiometric sensors for the determination of pioglitazone in some pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **2008**, *48*, 57.
[27] M. Aghaie, M. Giahi, H. Aghaie, M. Arvand, M. Pounaghdy, F. Yavari. New Fe(II) ion-selective electrode based on N-phenylaza-15-crown-5 as neutral carrier in PVC matrix. *Desalination* **2009**, *247*, 346.
[28] S. S. M. Hassan, M. A. Abou Ghalia, A-G.E. Amr, A. H. K. Mohamed. New lead(II) selective membrane potentiometric sensors based on chiral 2, 6-bis-pyridinecarboximide derivatives. *Talanta* **2003**, *60*, 81.
[29] S. S. M. Hassan, E. M. Elnemma, A. H. K. Mohamed. Novel potentiometric copper (II) selective membrane sensors based on cyclic tetrapeptide derivatives as neutral ionophores. *Talanta* **2005**, *66*, 1034.
[30] S. S. M. Hassan, M. A. Abou Ghalia, A-G. E. Amr, A. H. K. Mohamed. Novel thiocyanate-selective membrane sensors based on di-, tetra- and hexa-imidepyridine ionophores. *Anal. Chim. Acta* **2003**, *482*, 9.
[31] A. E. Amr, O. I. Abdel-Salam, A. Attia, I. Stibor. Synthesis of new potential bis-intercallators based on chiral pyridine-2,6-dicarboxamides. *Collect. Czech. Chem. C.* **1999**, *64*, 288.
[32] A. Carggs, G. J. Moody, J. D. R. Tomas. PVC matrix membrane ion-selective electrodes. *J.Chem. Educ.* **1974**, *51*, 541.
[33] R. P. BucK, E. Linder. Recommendations for nomenclature of ion-selective electrodes. *Pure Appl. Chem.* **1994**, *66*, 2527.

[34] D. Amman, E. Pretsch, W. Simon, E. Lindner, A. Bezegh, E. Pungor. Lipophilic salts as membrane additives and their influence on the properties of macro- and micro-electrodes based on neutral carriers. *Anal. Chim. Acta* **1985**, *171*, 119.
[35] P. Holy, W. E. Morf, K. Seiler, W. Simon, J. P. Vigneron. Enantioselective optrode membranes with enantiomer selectivity for (R)- and (S)-(1-phenylethyl)ammonium [(alpha-methylbenzyl)ammonium] ions. *Helv. Chim. Acta* **1990**, *73*, 1171.
[36] S. S. Hassan, W. H. Mohmoud, A. M. Othman. Ribonucleic acid as a novel ionophore for potentiometric membrane sensors of some transition metal ions. *Talanta* **1998**, *47*, 377.
[37] M. B. Gholivand, Y. Mozaffari. PVC-based bis(2-nitrophenyl)disulfide sensor for zinc ions. *Talanta* **2003**, *59*, 399.
[38] T. S. Ma, S. S. M. Hassan. *Organic Analysis Using Ion Selective Electrodes*. Academic Press: London, **1982**, Vols 1 & 2.
[39] Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda, S. Amemiya. Potentiometric selectivity coefficients of ion-selective electrodes, part1. Inorganic cations. *Pure Appl. Chem.* **2000**, *72*, 1851.
[40] J. C. Miller, J. N. Miller. *Statistics for Analytical Chemistry*. Ellis Horwood Ltd.: England, **1986**, 1st Ed. pp. 43, 53, 59, 189, 192.