



Physico-chemical properties and characterization of iron (II) electrochemical sensor based on carbon paste electrode modified with novel antimicrobial Carboxymethyl chitosan-graft-poly(1-cyanoethanoyl-4-acryloyl-thiosemcarbazide) copolymers

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ABSTRACT

Carbon paste electrode (MCPE) modified with novel Carboxymethyl chitosan/poly(1-cyanoethanoyl-4-acryloyl thiosemcarbazide) copolymers (CMCS-PCEATS) was prepared for determination of iron(II). The interaction between the modifier (CMCS-PCEATS) and iron(II) ions at the electrode surface was characterized using scanning electron microscope (SEM), the energy dispersive X-ray analyzer (EDX) and FTIR. Effect of different plasticizers (TCP, o-NPOE, FPNPE, DBP, DOP and DOS) and content of modifier were studied. The best performance was obtained with the electrodes plasticized with FPNPE (electrode I) and TCP (electrode II). Under the optimized conditions, the electrodes reveal Nernstian slopes of 30.21 ± 0.31 and 30.17 ± 42 mV decade⁻¹ over a wide concentration range from 1.0×10^{-7} to 1.0×10^{-1} mol L⁻¹ with a detection limit of 1.0×10^{-7} mol L⁻¹ at pH range from 3 to 8 for electrodes I and II. The paste is enough stable for 37 days without any measurable divergence in the potential. This method was successfully applied for potentiometric determination of iron (II) in pharmaceutical and water samples, and the results obtained agreed with those obtained with spectrophotometer and inductive coupled plasma (ICP). The thermal stability as well as the antimicrobial activity of the MCPE and MCPE-iron(II) were also studied. The results illustrated that MCPE-iron(II) was thermally stable over wide range of temperature than MCPE and it has higher antibacterial activity against *Streptococcus aureus*, *Salmonella SP.*, *Escherichia coli* and antifungal activity against *Candida albicans*.

1. Introduction

Iron is more distributed in nature, and it is one of the essential elements in biological and environmental systems. Iron plays an important role in diversity of cellular events and indeed no life form is possible without this element with a few possible exceptions in bacterial world [1,2], though it is a critical element for living systems. The gradual cumulation of iron leads to some diseases [3–6]. The increase of iron in body causes kidney and liver damage (hemochromatosis), but its deficiency leads to anemia [7–9]. Several methods for the determination of iron are available, including inductively coupled plasma–mass spectrometry [10,11], atomic absorption spectrometry [12,13] and emission spectrometry [14–16]. However, most of these methods require numerous time-consuming manipulation steps [17,18]. Potentiometric ion-selective electrodes (ISEs) have been extensively used in determination of ions in both pharmaceutical and water samples [19–22]. They also used in determination of anionic species such as

dichromate, perchlorate, oxalate ions [23–25] and widely used for the direct potentiometric determination of ion concentrations or ion activities in various samples. Their advantages are low cost, simple design, low detection limit, high accuracy and adequate selectivity [26–29]. Recently, the attention of researchers was directed to use voltammetric sensors based on a graphene–carbon paste electrode modified with imprinted and non-printed polymers for electrochemical determination of many drugs and elements potentiometrically. Carbon paste electrodes provide a renewable surface, low ohmic resistance and stable response; also they can be modified by nano-materials [30–32].

The goal of this work is to prove the ability of a good ionophore in the construction of carbon paste electrodes and the use of these modified electrodes as electrochemical sensors for Fe (II) determination. The characteristics and analytical performance of MCPES like influence of ionophore content, different plasticizers, pH range, effect of the interfering cations and temperature have been investigated. It is worth mention that we use novel Carboxymethyl chitosan-graft-poly(1-

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cyanoethanoyl-4-acryloyl thiosemicarbazide)co-polymers (CMCS-PCEATS) as an ionophore. Whereas Both CMCS and PCEATS separately have been reported as a better chelating agents towards several metal ions as Cu(II), Ni(II), Cd(II), Co(II), ...etc. [33–36]. Grafting of CMCS with 1-Cyanoethanoyl-4-acryloyl thiosemicarbazide gives CMCS-PCEATS co-polymers as a novel co-polymers that characterized by a higher chelating capacity due to the presence of different kinds of function groups (OH, COOH, NH₂, CONH, C=S, CN, ...) with a polar and hydrophilic characters that facilitate the chelation with the metal ions. In addition they have wide biological application. Since thiosemicarbazides and thiosemicarbazones are considered have higher biological applications they exhibits potential activity in the field of anticancer, antitubercular, anti HIV, antidepressant, antiviral, antifungal, antibacterial and anticonvulsant. Also they have parasiticidal activity against Plasmodium, Trypanosoma cruzi ... etc. [37,38]. These may be due to the presence of the S and N donors with its higher chelation interaction. On the other hand Carboxymethyl chitosan (CMCS) exceeds chitosan in its solubility in water and different organic solvents, in addition it is characterized by higher nontoxicity, biodegradability and biocompatibility it has antimicrobial activity towards different kinds of bacteria and fungi. Its antibacterial activity resulted from the ability to bind and disrupt the normal function of bacteria cell membrane thus promoting the leakage of intracellular components and prevent transportation of the nutrients into the cells [39–42] while, the inhibiting effect on fungi is due to the disturbance of the enzyme activities responsible for the growth criteria. Carboxymethyl chitosan and its derivatives were employed in many medical applications as artificial bone and skin, a moisture-retention agent, blood anticoagulant, wound dressing agent, and also in drug delivery systems, antioxidants [43]. In this work we prepared CMCS grafted with a monomer bearing thiosemicarbazide moiety as antimicrobial modifier for carbon paste electrode which can be used in determination of Iron (II) in pharmaceutical and water samples. The modified carbon paste electrode (MCPE) considered a promising as Iron (II) electrochemical sensor.

2. Experimental

2.1. Instrumentation and chemicals

All potentiometric measurements were performed at $25 \pm 1^\circ\text{C}$ using a digital Hanna pH/mV meter (model 211), scanning electron microscope (SEM) and the energy dispersive X-ray analyzer (EDX), were made in National research center, Egypt. The microanalysis were completed using FT-IR spectra were recorded on a Perkin-Elmer 1650 spectrometer ($4000\text{--}400\text{ cm}^{-1}$) in KBr pellets at the Micro analytical Center, Cairo University, Egypt. Silver-silver chloride double-junction reference electrode in conjugation with different ion selective electrodes were used, concentration of iron(II) determined using analytika Jena spectrophotometer (Germany) and ICP-OES PerkinElmer optima 7300DV American.

Graphite fine powder extra pure, Ammonium iron (II) sulfate $[(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$ were of analytical grade and purchased from Merck (Germany), o-nitro phenyl-octylether (o-NPOE) was supplied from Fluka. Dioctylphthalate (DOP), 2-florophenyl 2-nitrophenylether (FPNPE), Tricresylphosphate (TCP), dibutylphthalate (DBP) and dioctylsebacate (DOS) were supplied from BDH. Chloride salts of sodium, potassium, zinc, cadmium, cobalt, chromium, manganese, nickel and copper were used as interfering materials which were supplied from El Nasr Company.

Chitosan with a degree of deacetylation of 88% and molecular weight of 2.0×10^5 was purchased from Acros Organics, New Jersey, USA. Potassium persulfate was of analytical grade and was supplied from Merck Chemicals. Other reagents and solvents were of analytical grades from Aldrich and were used as received. 1-Cyanoethanoyl-4-acryloyl thiosemicarbazide (CEATS) was synthesized according to the method described previously [36]. Carboxymethyl chitosan (CMCS)

Table 1
Water samples location.

No. of sample	Type	Location
1	Drinking water	Elhager plant
2	Drinking water	Embaba plant
3	Drinking water	Nekla plant
4	Drinking water	Gziretelwrak plant

was prepared following the method reported in literatures [44,45].

2.2. Water and pharmaceutical samples

Water samples from different sources were collected and acidified by nitric acid, and analyzed for Fe(II) ions content by the MCPE and ICP method (Table 1).

Drug samples:

1. Marvit (syrup), each 5 mL contains 9 mg ferrous gluconate, manufactured by Marcyrl Pharmaceutical Industries–ElOubour City Cairo-Egypt.
2. Pharovit (syrup), each 100 mL contains 1.04 g ferrous gluconate (equivalent to 120 mg iron), manufactured by Pharaonia pharmaceutical spharopharma new Borg El Arab city Alexandria-A.R.E.

2.3. Preparation of carboxymethyl chitosan-graft-poly(1-cyanoethanoyl-4-acryloyl thiosemicarbazide) copolymers (CMCS-PCEATS)

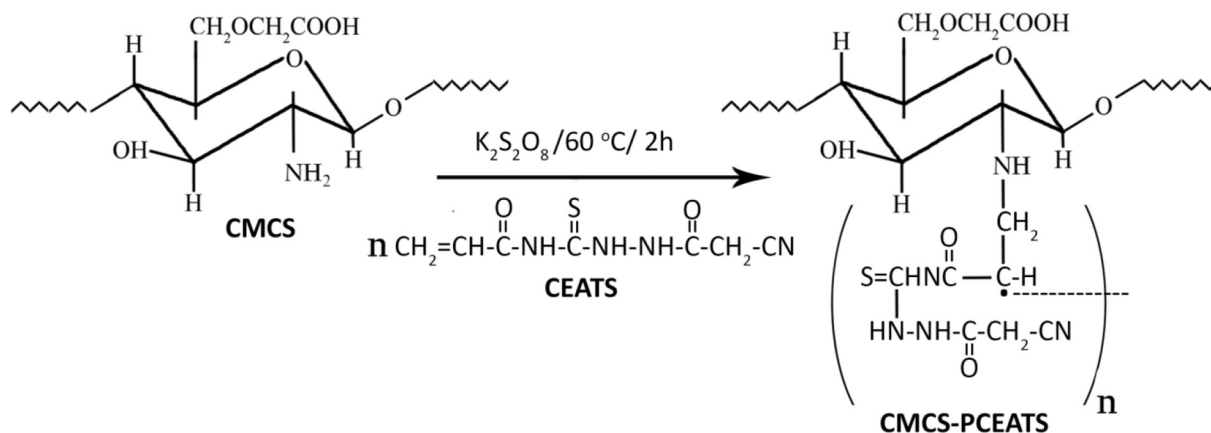
Calculated amount (0.5 g) of dry carboxymethyl chitosan was dissolved in double-distilled water in a three-necked round-bottom flask, with a 1:50 liquor ratio, followed by the addition of 2.64 g (0.25 mol L^{-1}) of the prepared 1-Cyanoethanoyl-4-acryloyl thiosemicarbazide (monomer). The flask was placed in a thermostat bath at 60°C . Nitrogen gas was passed for 30 min under stirring to remove the dissolved oxygen. Then 0.4 g ($3 \times 10^{-2}\text{ mol L}^{-1}$) of potassium persulfate (initiator) was slowly added to the flask to initiate graft copolymerization. After 2 h, the reaction mixture was poured on cold methanol to precipitate the grafted co-polymers, Carboxymethyl chitosan-graft-poly(1-cyanoethanoyl-4-acryloyl thiosemicarbazide), designated as (CMCS-PCEATS) (Scheme 1). After that, the grafted copolymers were separated by filtration and dried under vacuum at 50°C until constant weight was reached. Purification of the grafted copolymers from the homopolymer was achieved by extraction of the products with methanol using a soxhlet for 10 h [46]. The grafting percentage was determined from the equation $\%G = [(W_2 - W_1)/W_1] \times 100$ where W_1 denote the weight of initial carboxymethyl chitosan and W_2 denote the grafted copolymers after extraction with methanol, the calculated %G was 20%.

2.4. Preparation of the modified carbon paste electrodes (MCPEs)

Modified carbon paste electrodes (MCPEs) were prepared by thoroughly mixing different amounts of CMCS-PCEATS (5–15 mg), carbon powder (250 mg) and 100 μL plasticizer (TCP, DBP, DOP, DOS, o-NPOE or FPNPE) in a mortar and the resulted paste was used to fill the electrode body [47]. These sensors were used directly for potentiometric measurements without any preconditioning [48]. A new surface of the paste was obtained by squeezing more out, the surplus paste was wiped out and the freshly exposed surface was polished on a paper until the surface showed shiny appearance.

2.5. Potential measurement

All Electromotive force (EMF) measurements were carried out with the following assembly cell: Ag | AgCl | satd. KCl || sample solution |



Scheme 1. Schematic representation for CMCS-PCEATS preparation.

sensor (MCPE). EMF was plotted as a function of the negative logarithm of iron (II) ion concentration. The detection limit was taken at the point of intersection of the extrapolated linear segment of the calibration curve, the selectivity coefficients ($K_{\text{Fe}^{2+}, \text{B}}^{\text{pot}}$) were measured using the separate solution method using 0.001 mol L^{-1} of Fe(II) and interfering ions.

2.6. Determination of Fe(II) in water and pharmaceutical samples using MCPE

About 10 mL water or pharmaceutical samples were transferred to a 25 mL beaker and their content was determined via potentiometric calibration using MCPE as sensing electrode. This method was repeated several times in order to check the reproducibility and accuracy of the proposed method.

2.7. Spectrophotometric determination of Fe(II) in water samples

Acidify a water samples (1–4) with 2 mL conc HCl/100 mL sample at time of collection. Immediately withdraw a 50 mL portion of acidified sample and add 20 mL phenanthroline solution and 10 mL $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ solution with vigorous stirring. Dilute to 100 mL and measure the absorption within 5 to 10 min at wavelength 510 nm [49,50].

2.8. Surface analysis

Energy dispersive X-ray analyzer (EDX) and scanning electron microscope (SEM) were used for morphological analysis of the adsorbent at $8000\times$ magnifications for the electrode II before and after the interaction with iron (II) ions.

2.9. Antimicrobial measurements

Antibacterial activities were investigated using the agar well diffusion method. The activity of tested samples was studied against *Streptococcus aureus* (RCMB 010010) as Gram positive bacteria and against *Salmonella SP.* (RCMB 010043), *Escherichia coli* (RCMB 010052) as Gram negative bacteria. The reactivity of the tested sample against the microorganisms was determined by measuring the inhibition zone diameter (in mm). All compounds were prepared in DMSO, and DMSO was loaded as control. The test was performed three times for each bacterium culture: Ampicillin and Gentamicin were used as antibacterial standard drugs [51].

Antifungal activities were studied by screening the tested samples separately in vitro against *Candida albicans* (RCMB 05036) fungus on sabouraud dextrose agar plates. The culture of fungus was purified by the single spore isolation technique. The antifungal activity was studied

by agar well diffusion method [52]. Amphotericin B was used as antifungal standard drug.

3. Results and discussion

In recent decades, with increasing emphasis on health, safety, and the environment, the requirement for new detection techniques with high sensitivity and universality is strongly demanded for the assay of trace amounts of biological targets, such as small molecules, viruses, bacteria, evolved from electrochemical bioanalysis which exhibit many intrinsic advantages, such as a fast response, a low background, and high sensitivity. Many researchers apply potentiometric immunosensor for the determination of low-abundant biomolecules based on the change in the surface charge as a result of the antigen-antibody reaction which causes a shift in the electrical potential [53–55]. Extending for this approach we prepared electrochemical biosensor based on modified carbon paste electrode useful for biological application. Modification of carbon paste electrode with functionalized biopolymers as Carboxymethyl chitosan/poly(1-cyanoethanoyl-4-acryloyl thiosemicarbazide) copolymers construct electrode with higher chelation capacity to be used for determination of iron (II) metal in water and pharmaceutical samples. The effect of paste composition, working range, selectivity, pH of the media, life time of the paste, thermal stability and the antimicrobial activity of the MCPE were investigated in this study.

3.1. Characterization of the prepared CMCS-PCEATS co-polymers

3.1.1. FTIR analysis

Fig. 1 showed the FTIR spectrum of CMCS, CEATS (monomer) and CMCS-PCEATS co-polymers. The spectrum of CMCS showed four characteristic peaks at 1157 , 1071 , 1035 and 878 cm^{-1} corresponding to the polysaccharide structure, strong broad band around 3444 cm^{-1} related to the stretching vibration of NH_2 and OH groups, broad band at 1636 cm^{-1} related to symmetrical vibration of the $-\text{COO}^-$ group overlapped with the bending vibration of the NH_2 group and at 1435 cm^{-1} corresponding to asymmetric vibration of $-\text{COO}^-$ groups overlapped with the bending vibration of CH_2 groups. The FTIR spectrum of CEATS showed the following absorption peaks: at 3443 cm^{-1} corresponding to NH stretching vibration, the peaks at 3181 , 3124 , 3052 cm^{-1} are characteristic to the $\text{C}=\text{C}$ group, while the peak at 2891 cm^{-1} is related to the CH_2 groups. The absorption bands appeared at 2362 , 2260 cm^{-1} are corresponding to the $\text{C}\equiv\text{N}$ group, while that at 1654 cm^{-1} is corresponding to $\text{C}=\text{O}$ of the amide group and that at 1482 , 1378 cm^{-1} are due to $\text{C}=\text{S}$ group. The $\text{N}-\text{C}-\text{S}$ absorption band appeared at 680 , 526 cm^{-1} . On the other hand, the spectrum of CMCS-PCEATS showed observable increase in the intensity of the peaks at 3426 (NH_2), 2925 (CH_2) and 2361 , 2336 ($\text{C}\equiv\text{N}$) as a result of grafting process. The broad band at 1644 cm^{-1} is attributed to

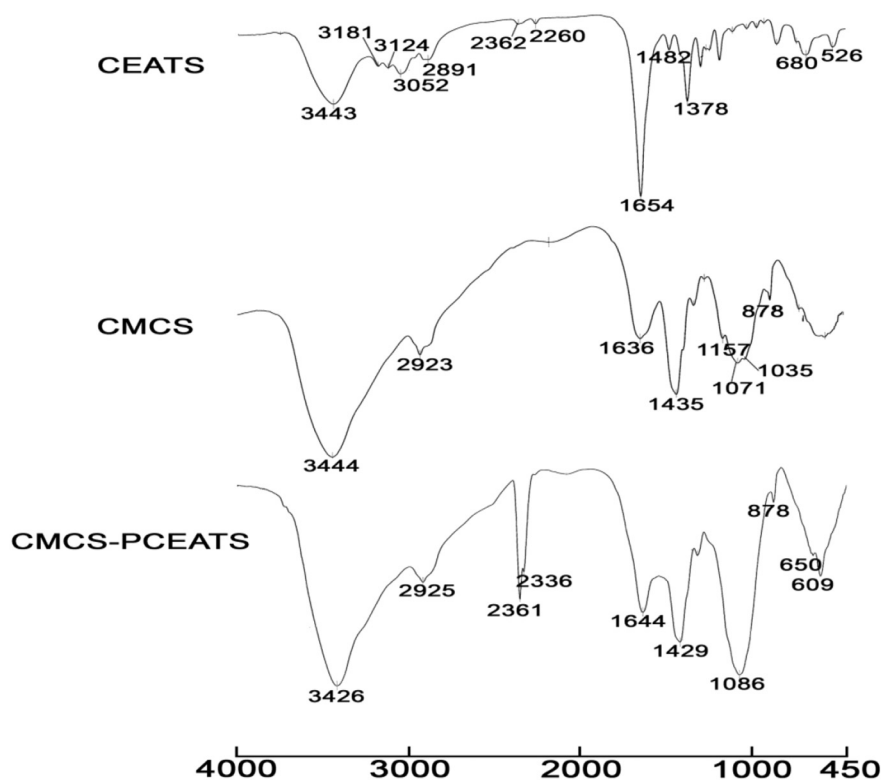


Fig. 1. FTIR spectrum of CEATS, CMCS and CMCS-PCEATS co-polymers.

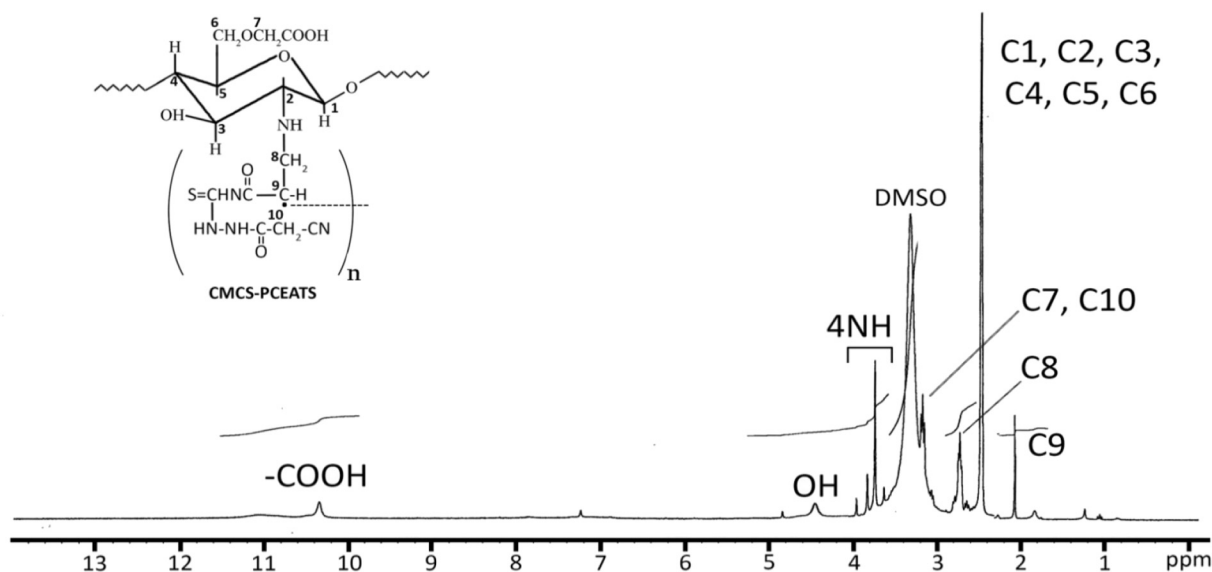


Fig. 2. ^1H NMR spectrum of CMCS-PCEATS co-polymers.

the stretching vibration of C=O groups overlapped with the bending vibration of NH_2 group and The peak at 1429 cm^{-1} is characterized to C=S group while that at $650, 609\text{ cm}^{-1}$ are due to N-C-S group. Moreover, the absence of the peaks at $3181, 3124, 3052\text{ cm}^{-1}$ for C=C group from the CMCS-PCEATS spectrum confirms the introduction of the CEATS molecules onto the CMCS backbone. The above data confirm the successful formation of the CMCS-PCEATS co-polymers.

3.1.2. ^1H NMR analysis

^1H NMR spectrum of CMCS-PCEATS co-polymers showed the following signals: the signal at $\delta = 2.066\text{ ppm}$ related to -CH proton of C9 of the pendent group (t, H, -CH) while the signals at $2.496\text{--}2.706$ is

corresponding to the CMCS protons at C1, C2, C3, C4, C5, C6 of the pyranose ring. While the signals at $2.743\text{--}2.805\text{ ppm}$ is related to the protons of C8 of the pendent group (d, 4H, 2CH_2), and that at $\delta = 3.151\text{--}3.190\text{ ppm}$ is due to the protons at C7 and C10 (s, 4H, 2CH_2). Moreover there is a characteristic signal at 3.634 ppm related to the NH group (s, 1H, NH) of the CMCS glucose ring and 3 signals at $\delta = 3.742\text{--}3.962\text{ ppm}$ related to the 3 NH of the CEATS moiety (3 s, 3H, 3NH). On the other hand the appeared signals at 4.250 and 10.342 ppm are related to the OH and -COOH protons respectively. These data confirm the structure of the prepared CMCS-PCEATS co-polymers as shown from Fig. 2.

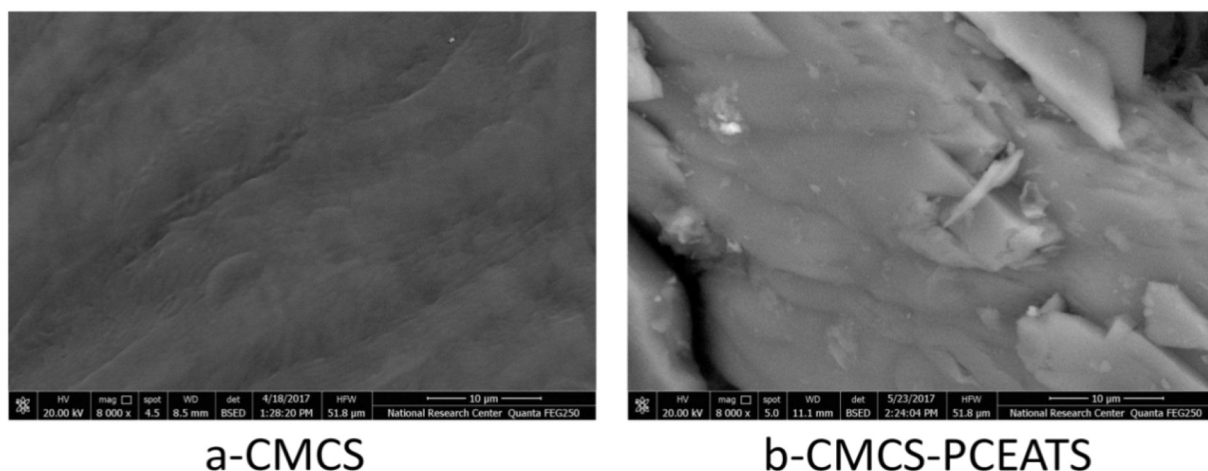


Fig. 3. SEM images of CMCS and CMCS-PCEATS co-polymers.

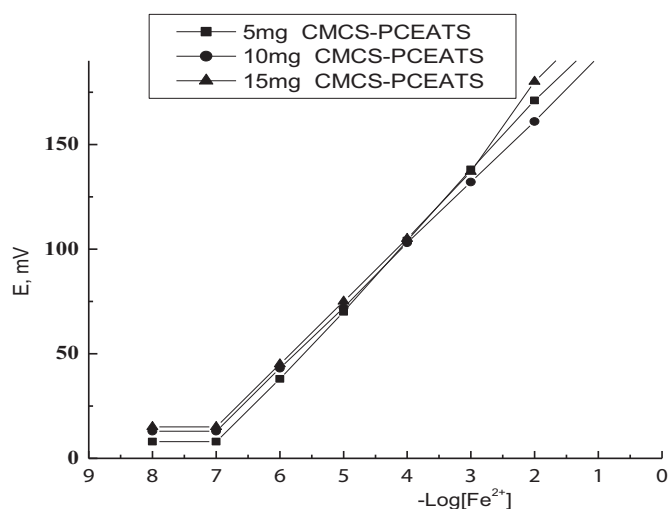


Fig. 4. Effect of CMCS-PCEATS content on potential response of MCPE, electrode II for determination of Fe(II) ions in ammonium acetate buffer (pH = 3.3).

Table 2

The plasticizer types effect on the performance of the MCPE.

Plasticizer	Slope mV decade ⁻¹	Correlation coefficient, R ²	Linear range mol L ⁻¹
DBP	32.6	0.994	1.0×10^{-6} – 1.0×10^{-2}
DOP	28.8	0.9999	1.0×10^{-6} – 1.0×10^{-2}
DOS	28.8	0.9997	1.0×10^{-6} – 1.0×10^{-2}
TCP	30.17	0.9999	1.0×10^{-7} – 1.0×10^{-1}
FPNPE	30.21	0.9999	1.0×10^{-7} – 1.0×10^{-1}
o-NPOE	31.6	0.9994	1.0×10^{-6} – 1.0×10^{-2}

3.1.3. Scanning electron microscopy observations of the grafted copolymers (SEM)

The scanning electron micrographs of CMCS and CMCS-PCEATS copolymers were shown in (Fig. 3 a, b). The surface morphology of the CMCS was modified in the graft co-polymer, wherein distinct morphological differences were clear in their surface topography, whereas grafting process resulted in co-polymer with clustered irregular structure.

3.2. Effect of CMCS-PCEATS co-polymers content

Paste composition is an important parameter for the electrode. When the amount of the electro active material in the matrix is

sufficient to attain reasonable ionic exchange, chemical equilibrium at the membrane or electrode/solution interface will be reliable for the electrode potential. For this target, the electrodes plasticized with TCP were prepared containing different amounts of the ionophore, 10 and 15 mg of CMCS-PCEATS which exhibit the slopes 32.5, 30.17 and 32.8 mV decade⁻¹, respectively. It was clear that, the optimum CMCS-PCEATS content was found to be 10 mg which give the best sensitivity, with Nernstian slope of 30.17 mV decade⁻¹, with a good correlation coefficient and very low detection limit 1.0×10^{-7} mol L⁻¹ which was calculated by the extrapolating of the two segment of the calibration curve Fig. 4.

3.3. Effect of plasticizers type on MCPE performance

The nature of the plasticizer is an essential feature that affects the characteristics of the sensors. It is important for the plasticizer to have definite properties, such as low vapor pressure, high lipophilicity, high molecular weight and high capacity to dissolve the substrate and other additives present in the matrix to be used in the sensors. In this study the effect of the plasticizer type on the performance of the sensors was inspected using six plasticizers namely o-NPOE, DBP, TCP, DOP, DOS and FPNPE as shown in (Table 2). It was found that the electrodes plasticized with FPNPE and TCP showed best potentiometric responses, i.e. best Nernstian slope, sensitivity and wide linearity range of the calibration plots, this can be attributed to its relatively high molecular weight and high dielectric constant. The subsequent studies were carried out on CPE modified with 10 mg CMCS-PCEATS and plasticized with FPNPE (electrode I) or TCP (electrode II).

3.4. Surface characterization of the MCPE

The surface analysis for MCPE, electrode II was obtained via EDX and SEM images which were used to identify the different zones of the electrode surface before and after the reaction with Fe(II) ions. The micrographs of electrode II showed more spaces throughout electrode paste surface (Figs. 5, 6 (a)) which was filled with iron ions as a result of coordination between the iron ions and the CMCS-PCEATS ionophore after soaking the electrode II in iron solution (Figs. 5, 6 (b)). These data is supported by FTIR spectra, The recorded FTIR spectrum for MCPE before soaking in Fe(II) solution showed in addition to the characteristic absorption bands for CMCS-PCEATS that explained above, an additional band at 1613, 1587 and 970 cm⁻¹ due to the additive graphite nucleus. Soaking of MCPE in Fe (II) solution resulted in coordination between the functionalized MCPE and Fe(II) ions (Scheme 2), which resulted in shift in the position of many absorption bands of the free MCPE, as the absorption peak at 1647 cm⁻¹ (C=O overlapped with

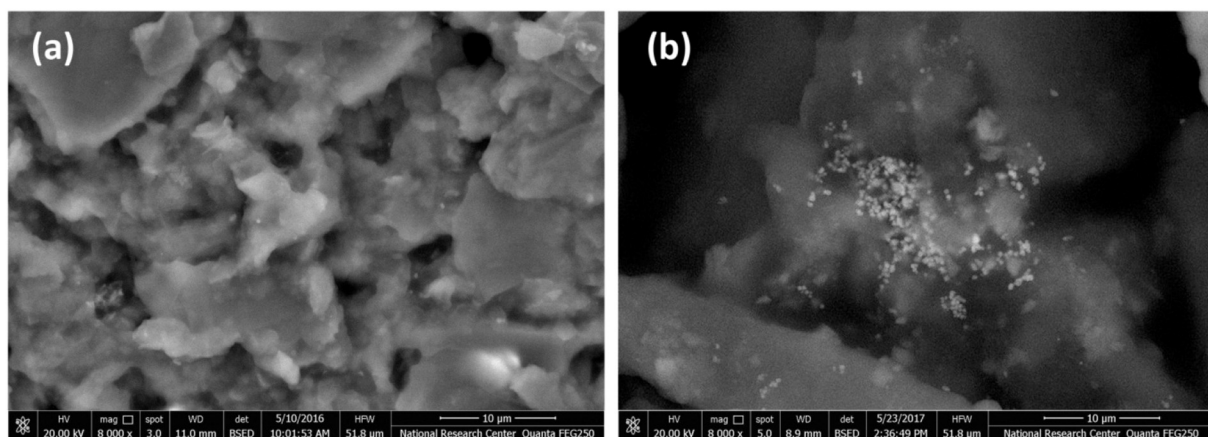


Fig. 5. SEM images for electrode II surface (a) before and (b) after soaking in $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Fe(II)}$ ions.

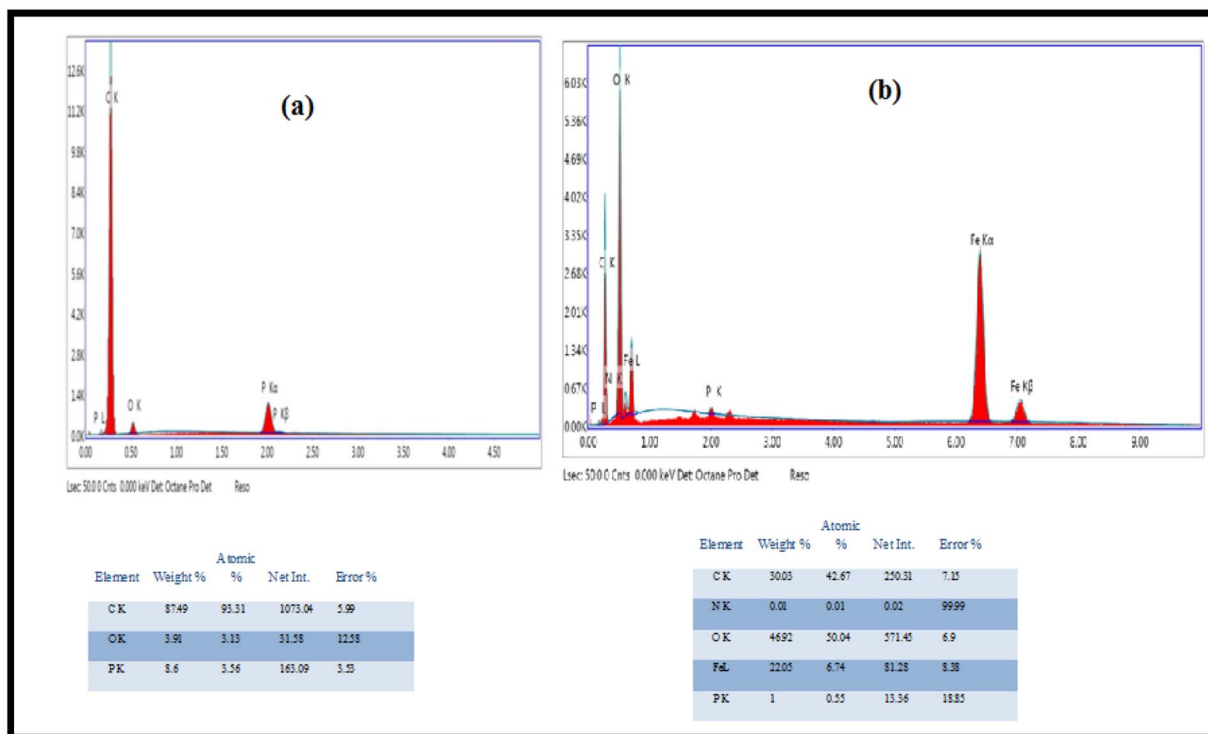


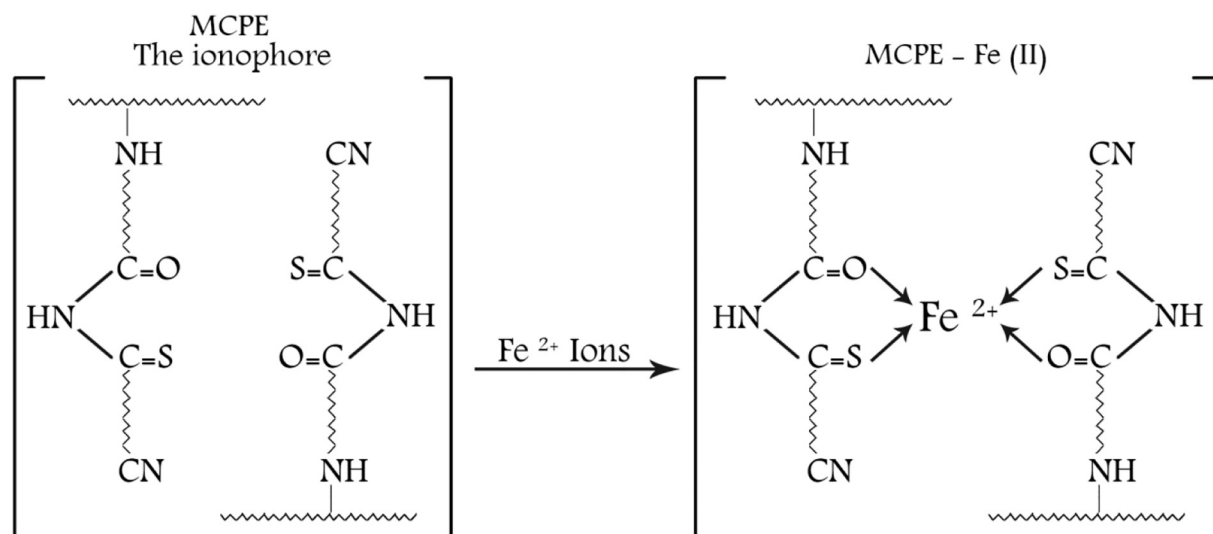
Fig. 6. EDX of electrode II (a) before and (b) after soaking in $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Fe(II)}$ ions.

NH_2 bending) is shifted to 1637 cm^{-1} up on chelation with the metal ion. The appearance of a new absorption peaks at 1380 (C=S) , 600 (N-C-S) and $2044 \text{ cm}^{-1} \text{ (C=N)}$ in the spectrum of MCPE-Fe(II) confirm the chelation between the MCPE function groups and the Fe (II) ions as indicated from Fig. 7.

3.5. Thermogravimetric analysis (TGA) of MCPE and MCPE-Fe(II)

The effect of the incorporated metal onto the thermal stability of the prepared MCPE was studied by recording the thermogravimetric analyses for the MCPE (electrode II) before and after soaking in Fe(II) ion solution. (Fig. 8) Showed the thermograms of both free MCPE and MCPE-Fe(II). The results showed higher thermal stability of the MCPE-Fe(II) than the free MCPE, the thermal analysis was recorded at $10 \text{ }^\circ\text{C min}^{-1}$ heating rate, under constant nitrogen flow rate of 30 mL min^{-1} . The thermogram of the free MCPE exhibited weight loss in four clear stages. The first weight loss stage occurred between 37 and $201 \text{ }^\circ\text{C}$ could be resulted from evaporation of the moisture adsorbed on

the MCPE surface it lost about 9.5% of its original weight. An appreciable weight loss about 21.23% occurred in the temperature range from 201 to $274 \text{ }^\circ\text{C}$ in the second weight loss stage which indicated the induced cyclodehydration reaction of the thiohydrazide linkage of the CMCS-PCEATS into highly stable 1,3,4-thiadiazole ring by losing water on heating [35,56] and the dehydration between the carboxylic groups of the CMCS to form anhydride. In the third stage the MCPE lost about 12.1% of its weight at the temperature range from 247 to $353 \text{ }^\circ\text{C}$ could be due to the decarboxylation and the elimination of the NH_2 , in the form of NH_3 , from the CMCS backbone. About 13% weight loss occurred at temperature range from 353 to $600 \text{ }^\circ\text{C}$ resulted from the degradation the MCPE possessing the 1,3,4-thiadiazole ring and decomposition of graphite to CO_2 gas evolved. At this stage the remaining weight of the free MCPE was about 45% of its original weight percent. On the other hand, the thermogram of the MCPE-Fe(II) showed only one degradation step in the temperature range from 9 to $334 \text{ }^\circ\text{C}$ with weight loss of 31% could be related to the removal of moisture, CO_2 and NH_2 , the remaining weight was 69%, thus no more degradation was



Scheme 2. Schematic representation of the coordination between MCPE and Fe(II) ion.

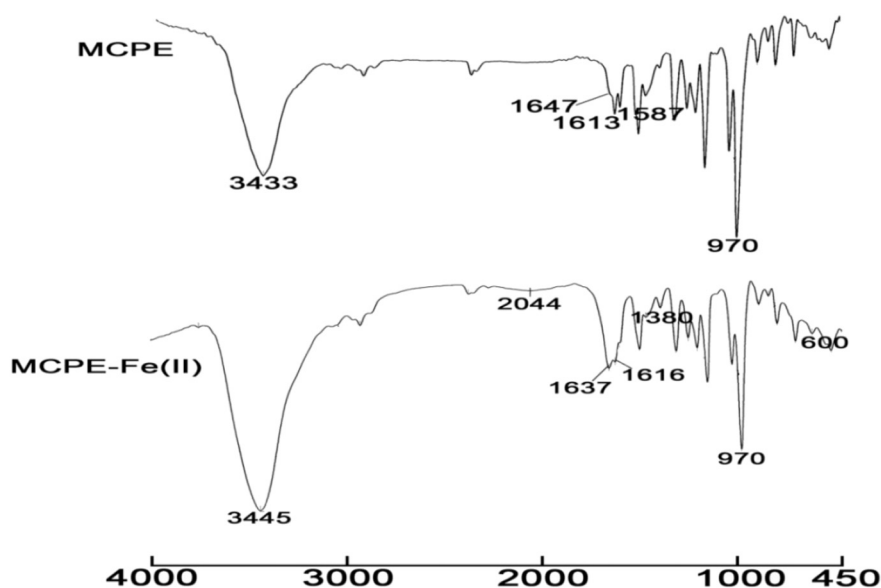


Fig. 7. FTIR spectrum of electrode II before and after soaking in $1.0 \times 10^{-3} \text{ mol L}^{-1}$ Fe(II) ions.

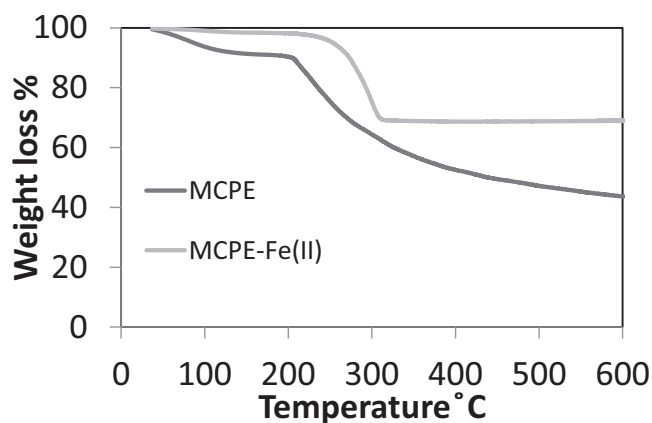


Fig. 8. Typical TG thermograms patterns of MCPE (electrode II) before and after soaking in $1.0 \times 10^{-3} \text{ mol L}^{-1}$ Fe(II) ions, the thermograms were recorded in nitrogen, at a heating rate of $10 \text{ }^\circ\text{C min}^{-1}$ and under a gas flow rate of 30 mL min^{-1} .

observed above $334 \text{ }^\circ\text{C}$, since chelation with the metal prevents the cyclodehydration reaction of the thiohydrazide groups which indicated the higher stabilizing effect of chelation on the degradation process as appeared from the higher initial decomposition temperature of the MCPE-Fe(II) ($245 \text{ }^\circ\text{C}$) than that of the free MCPE ($201 \text{ }^\circ\text{C}$). This supports the stabilizing effect of the metal on the thermal degradation of the MCPE, since chelation with the Fe(II) metal increased the order of the polymeric chains and thus decreased the thermal degradation.

3.6. Effect of pH

The effect of pH on the potential response was studied by use of 1.0×10^{-2} and $1.0 \times 10^{-4} \text{ mol L}^{-1}$ of Fe(II) ion solutions over the pH range of 2–12. Adjust the pH with small volumes of HCl and NaOH. The results obtained are shown in Fig. 9, which indicated that the sensors show a better response and extended linearity at acidic to slightly alkaline pH (3–8). At high alkaline medium the potential sharply decreased, due to the formation of some hydroxy complexes of Fe(II) ions in solution.

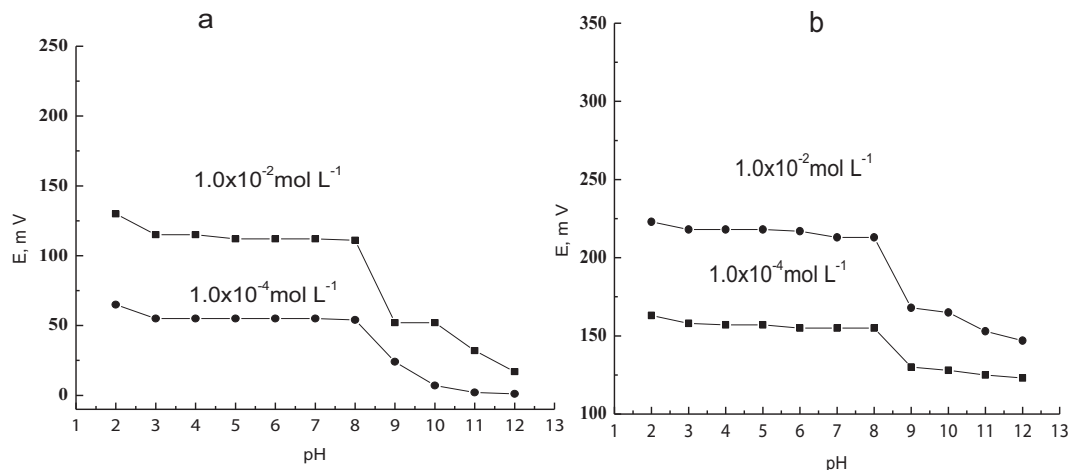


Fig. 9. The influence of pH on the performance of (a) electrode I and (b) electrode II for determination of Fe(II) ion.

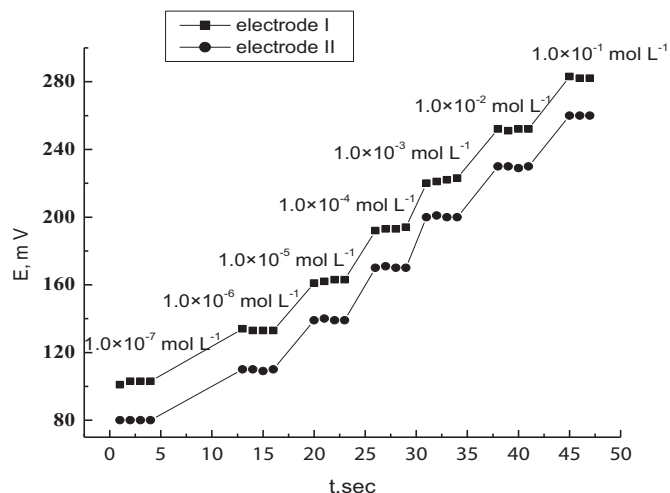


Fig. 10. Dynamic response time of electrode I and II for determination of Fe(II) ion.

3.7. Effect of temperature

The thermal stability of the electrodes is examined by testing the calibration curves at different solutions temperature range (10–60 °C). The standard cell potentials (E° cell) were determined at different temperatures from the respective calibration plots as the

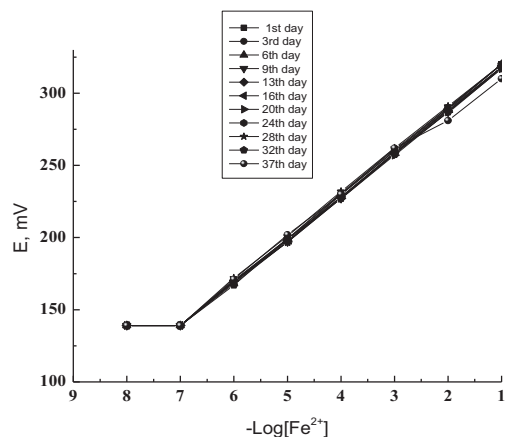


Table 3
Potentiometric selectivity coefficients of some interfering ions using SSM.

Interfering species	Log $K_{Fe^{2+}, B}^{pot}$	
	Electrode I	Electrode II
K^+	- 3.3	- 4.37
Na^+	- 3.4	- 3.77
Cr^{3+}	- 0.33	- 1.03
Mn^{2+}	- 1.66	- 2.93
Co^{2+}	- 1.7	- 2.2
Ni^{2+}	- 1.77	- 2.53
Zn^{2+}	- 1.86	- 1.03
Cd^{2+}	- 1.77	- 2.6
Cu^{2+}	- 1.6	- 1.03

intercepts of these plots at $pFe(II) = 0$, and were used to estimate the isothermal temperature coefficient (dE°/dt) of the cell with the aid of the following equation [57].

$$E^{\circ} = E^{\circ}(25) + [(dE^{\circ}/dt)](t - 25)$$

Plot of E° cell versus $(t-25)$ produced a straight line of slope which was taken as the isothermal temperature coefficient with values equals to 2.9×10^{-4} , 2.5×10^{-4} V/°C for electrodes I and II, respectively. The small values of (dE°/dt) electrodes revealed the high thermal stability of the proposed electrodes within the investigated temperature range.

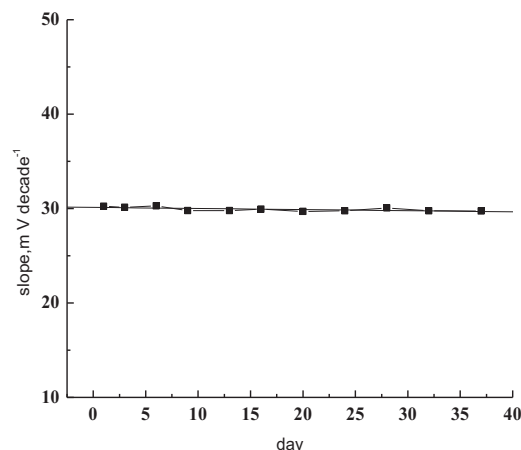


Fig. 11. Life time of the MCPE electrode for determination of Fe(II) ion.

Table 4
Intra- and inter- days precision for the determination of Fe(II) ions in real water samples using the proposed MCPES.

[Fe ²⁺]	Taken, mg mL ⁻¹	Found, mg mL ⁻¹		Recovery %		SD		RSD%	
		Intraday	Interday	Intraday	Interday	Intraday	Interday	Intraday	Interday
Electrode I	0.0558	0.0556	0.0570	99.64	102.15	0.0015	0.00061	2.69	1.07
pure	0.00558	0.00571	0.00565	102.33	101.25	0.00025	0.0000776	4.37	1.37
sample(1)	0.2500	0.2502	0.2455	100.08	98.2	0.0017	0.0017	0.68	0.69
Electrode II	0.0558	0.0538	0.0543	96.41	97.3	0.00219	0.0023	4.07	4.23
pure	0.00558	0.005771	0.0058	103.4	105	0.000274	0.00009	4.7	1.55
sample (1)	0.25	0.2483	0.2456	99.32	98.24	0.0021	0.0032	0.84	1.30

Table 5
Determination of Fe (II) ion in water samples using electrode I and reported methods.

Sample no.	CPE (electode 1)			ICP				Spectrophotometry			
	Found, mg L ⁻¹	SD	RSD %	Found, mg L ⁻¹	SD	RSD %	t-test	Found, mg L ⁻¹	SD	RSD %	t-test
1	0.251	0.0008	0.32	0.249	0.003	1.2	1.633	0.2504	0.002	0.79	1.84
2	0.107	0.0006	0.56	0.1067	0.0006	0.56	1.225	0.1065	0.001	0.94	1.53
3	0.215	0.0008	0.37	0.216	0.0013	0.46	1.884	0.2157	0.001	0.46	2.04
4	0.157	0.0006	0.38	0.1573	0.002	1.27	0.900	0.1576	0.002	1.3	2.33

Table 6
Determination of Fe (II) ion in pharmaceutical samples using electrode I.

Pharmaceutical samples	Taken, mg L ⁻¹	CPE (electode 1)				
		Found, mg L ⁻¹	Recovery%	SD	RSD	t-test
Marvit	206.46	206.23	99.88	0.488	0.236	1.144
Pharovit	1200	1200.6	100.05	0.55	0.046	2.449

3.8. Response time

One of the important factors for evaluation of the analytical applicability of ion-selective electrodes is the response time. The practical response time has been measured by changing solutions with different Fe(II) concentrations from the lower 1.0×10^{-7} mol L⁻¹ to the higher concentration 1.0×10^{-1} mol L⁻¹. The actual potential against time traces is shown in (Fig. 10). As it is seen, the electrodes attain the equilibrium response in a very short time of about 5 s and this can be attributed to the selection of the best content of ionophore and plasticizer.

3.9. Lifetime of the paste

One of the most important issues is to follow the response of the electrodes I and II at different regular intervals to check the reproducibility and lifetime of the sensors (Fig. 11). It is indicated from the figures that the paste has life time up to 37 days without significant change from Nernstian slope.

3.10. Selectivity of ion sensing electrodes

The selectivity is an essential characteristic of a proposed MCPE electrode that delineates the extent to which the electrodes may be used in the determination of Fe(II) ion in the presence of other ions and extent of utility of any sensor in real sample measurement [58,59].

The selectivity coefficient values were determined by the separate solution method (SSM) [60] using the following equation:

$$\log K_{Fe^{2+},B}^{pot} = (E_B - E_{Fe^{2+}}) \setminus S + (1 - Z_B Z_{Fe^{2+}}) \log a_{Fe^{2+}}$$

$E_{Fe^{2+}}$ and E_B are the potentiometric response of $a_{Fe^{2+}}$ and a_B ,

respectively, $a_{Fe^{2+}}$ is the Fe(II) ion activity (1.0×10^{-3} mol L⁻¹) and a_B is the activity of an interfering ion (1.0×10^{-3} mol L⁻¹). The single ion activities were calculated by the extended Debye-Hückel equation [61], S is the Nernstian slope, Z_B and $Z_{Fe^{2+}}$ are the charge of the interfering ions and Fe(II), respectively. The values of selectivity coefficient are sufficiently smaller than 1.0 indicating that the present sensor are more selective to Fe(II) ion than all the interfering ions, as shown in Table 3.

3.11. Intra- and inter-day determination of Fe (II) ion

The applicability and validity of the proposed electrodes and repeatability of the results obtained were examined by carrying out four replicate experiments on various concentrations of Fe(II) ions in different water samples, (Table 4) shows the values of the inter- and intraday relative standard deviations for various concentrations of the samples, obtained from experiments carried out over a period of four days (inter-day) or within the same day (intra-day). The small values of the relative standard deviations indicate the reasonable repeatability of the proposed MCPE which are successfully applied to determine Fe (II) ions in pure water samples.

4. Analytical application

The proposed electrodes were used for the determination of iron (II) ion concentration in water samples (1–4) and pharmaceutical samples (Marvit and Pharovit) using potentiometric method as shown in (Tables 5 and 6). The samples were prepared as mentioned above in Sections (2.6 and 2.7) to be analyzed. The results obtained were also compared with those from spectrophotometric and ICP analysis. It is clear from the values in (Table 5) that there were a good agreement with those obtained by proposed electrodes and reported methods. Hence, the sensors can be successfully employed for the estimation of iron (II) ions.

Tabulated t values at 95% confidence level is 2.447 ($n = 6$).

Tabulated t values at 95% confidence level is 2.571 ($n = 5$).

5. Antimicrobial assay

Table 7 showed the antimicrobial investigation of CMCS, MCPE and MCPE-Fe(II) samples against *S. aureus* as Gram-positive bacteria and *S. SP*, *E. coli* as Gram-negative bacteria. All the prepared compounds

Table 7
Inhibition indices of CMCS, MCPE and MCPE-Fe(II) against *S. aureus*, *S. SP*, *E. coli* and *C. albicans*.

Samples	Tasted bacteria		Tasted fungi	
	<i>S. aureus</i> (RCMB 010010)	<i>S. SP</i> (RCMB 010043)	<i>E. coli</i> (RCMB 010052)	<i>C. albicans</i> (RCMB 05036)
	Inhibition zone (mm)		Inhibition zone (mm)	
CMCS	8.4 ± 0.25	11.2 ± 0.58	13.2 ± 0.36	14.5 ± 0.25
MCPE	11.3 ± 0.58	12.5 ± 0.25	14.7 ± 0.19	15.8 ± 0.25
MCPE -Fe(II)	12.5 ± 0.19	14.6 ± 0.25	15.1 ± 0.25	16.7 ± 0.19
Ampicillin	23.2 ± 0.12	–	–	–
Gentamicin	–	17.0 ± 0.58	19.0 ± 0.63	–
Amphotericin B	–	–	–	25.1 ± 0.12

Table 8
Comparative studies between the proposed Potentiometric electrodes and previously published ion-selective electrodes.

Ionophore used	Linear range mol L ⁻¹	Slope mV decade ⁻¹	LOD mol L ⁻¹	pH	Response time (s)	Method	
Carboxymethyl chitosan/poly (1-cyanoethanoyl-4-acryloyl thiosemicarbazide)	1.0 × 10 ⁻⁷ –1.0 × 10 ⁻¹	30.21 ± 0.31	& 30.17 ± 0.42	1.0 × 10 ⁻⁷	3–8	5	Present work modified CPE
Rhodamine-dimethyliminocinnamyl RC	2.0 × 10 ⁻⁶ –3.0 × 10 ⁻⁴	29.4	1.6 × 10 ⁻⁷	1.5–6.5	10	PVC membrane [62]	
chiral 2,6-bis-(carboxamide methyl ester)pyridine derivative	6.0 × 10 ⁻⁶ –1.0 × 10 ⁻³	31.5 ± 0.5	1.14 × 10 ⁻⁷	5–9	30	PVC membrane [63]	
N-Phenylaza-15-Crown-5 (NPA15C5)	1.0 × 10 ⁻⁶ –1.0 × 10 ⁻²	29.2 ± 0.6	4.0 × 10 ⁻⁶	5–7	15	PVC membrane [64]	
2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) with tetraphenylborate (TPB)	1.0 × 10 ⁻⁷ –1.0 × 10 ⁻³	31.9 ± 0.2	1.0 × 10 ⁻⁷	3.1–7.2	10	PVC membrane [65]	
2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) with phosphotungstic acid (PTA)	1.0 × 10 ⁻⁶ –1.0 × 10 ⁻²	30.5 ± 0.1	1.0 × 10 ⁻⁶	2.5–7	10	PVC membrane [65]	
Carboxylated PVC	1.0 × 10 ⁻⁵ –1.0 × 10 ⁻²	32.7 ± 0.1	1.0 × 10 ⁻⁵	2.2–7	8	PVC membrane [65]	

exhibited higher antibacterial activity on both kinds of bacteria specially on Gram-negative bacteria as indicated from the values of inhibition zone diameters which ranging from 8.4 ± 0.25 to 12.5 ± 0.19 mm against *S. aureus* corresponding to 23.2 ± 0.12 mm for the standard antibiotic Ampicillin and ranging from 11.2 ± 0.58 to 14.6 ± 0.25 mm against *S. SP* close to that recorded for the standard drug Gentamicin 17.0 ± 0.58 mm, while the recorded inhibition zone diameter of the tested samples against *E. coli* was ranging from 13.2 ± 0.36 to 15.1 ± 0.25 corresponding to 19.0 ± 0.63 for the standard Gentamicin. The different effect on Gram-positive bacteria and Gram-negative bacteria was attributed to different microorganism cell wall structure. Whereas, Gram-positive bacterial cell wall is fully composed of peptidoglycan layer with choice acid while the cell wall of Gram-negative bacteria is composed of thin layer of peptidoglycan and outer membrane of lipopolysaccharide, lipoprotein and phospholipids.

On the other hand, studying the antifungal activity against *C. albicans* showed that CMCS, MCPE and MCPE-Fe(II) have potent inhibition effect on fungus growth as observed from the recorded values of inhibition zone diameters that ranging from 14.5 ± 0.25 to 16.7 ± 0.19 mm for the investigated samples corresponding to 25.1 ± 0.12 mm for the standard antibiotic Amphotericin B. The observed higher antimicrobial activity of the modified carbon paste electrode makes it very useful in removal of microorganisms from recycled waste water and tanks water in addition to iron determination, this extend the field of electrode biological applications.

6. Comparison between the proposed MCPE and other published ion-selective electrodes

The slope, linear range, detection limit, response time and working

pH of the previous works done in iron II with the proposed electrodes are compared in Table 8.

7. Conclusion

The proposed sensors carbon paste electrodes modified with CMCS-PCEATS co-polymers can successfully be applied in the potentiometric determination of Fe(II) ions. The described method was successfully applied for the determination of Fe(II) ions in water and pharmaceutical samples with high recovery in comparison with spectrophotometric and ICP methods. This study gives the ability to prepare thermally stable modified carbon paste electrode with higher performance and good sensitivity for iron (II) determination. Moreover, the prepared electrodes exhibited higher antimicrobial activity towards fungi and different kinds of bacteria, the reason for their advantage in many biological applications.

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