

Miniaturized PVC Sensors for Determination of Fe^{2+} , Mn^{2+} and Zn^{2+} in Buffalo-Cows' Cervical Mucus Samples

Ahmed S. Fayed, Umima M. Mansour

Abstract—Three polyvinyl chloride membrane sensors were developed for the electrochemical evaluation of ferrous, manganese and zinc ions. The sensors were used for assaying metal ions in cervical mucus (CM) of Egyptian river buffalo-cows (*Bubalus bubalis*) as their levels vary dependent on cyclical hormone variation during different phases of estrus cycle. The presented sensors are based on using ionophores, β -cyclodextrin (β -CD), hydroxypropyl β -cyclodextrin (HP- β -CD) and sulfocalix-4-arene (SCAL) for sensors 1, 2 and 3 for Fe^{2+} , Mn^{2+} and Zn^{2+} , respectively. Dioctyl phthalate (DOP) was used as the plasticizer in a polymeric matrix of polyvinylchloride (PVC). For increasing the selectivity and sensitivity of the sensors, each sensor was enriched with a suitable complexing agent, which enhanced the sensor's response. For sensor 1, β -CD was mixed with bathophenanthroline; for sensor 2, porphyrin was incorporated with HP- β -CD; while for sensor 3, oxine was the used complexing agent with SCAL. Linear responses of 10^{-7} - 10^{-2} M with cationic slopes of 53.46, 45.01 and 50.96 over pH range 4-8 were obtained using coated graphite sensors for ferrous, manganese and zinc ionic solutions, respectively. The three sensors were validated, according to the IUPAC guidelines. The obtained results by the presented potentiometric procedures were statistically analyzed and compared with those obtained by atomic absorption spectrophotometric method (AAS). No significant differences for either accuracy or precision were observed between the two techniques. Successful application for the determination of the three studied cations in CM, for the purpose to determine the proper time for artificial insemination (AI) was achieved. The results were compared with those obtained upon analyzing the samples by AAS. Proper detection of estrus and correct time of AI was necessary to maximize the production of buffaloes. In this experiment, 30 multiparous buffalo-cows were in second to third lactation and weighting 415-530 kg, and were synchronized with OVSynch protocol. Samples were taken in three times around ovulation, on day 8 of OVSynch protocol, on day 9 (20 h before AI) and on day 10 (1 h before AI). Beside analysis of trace elements (Fe^{2+} , Mn^{2+} and Zn^{2+}) in CM using the three sensors, the samples were analyzed for the three cations and also Cu^{2+} by AAS in the CM samples and blood samples. The results obtained were correlated with hormonal analysis of serum samples and ultrasonography for the purpose of determining of the optimum time of AI. The results showed significant differences and powerful correlation with Zn^{2+} composition of CM during heat phase and the ovulation time, indicating that the parameter could be used as a tool to decide optimal time of AI in buffalo-cows.

Keywords—PVC sensors, buffalo-cows, cyclodextrins, atomic absorption spectrophotometry, artificial insemination, OVSynch protocol.

I. INTRODUCTION

ION-selective electrodes (ISEs) have long been used for determination of metal ions in water and biological samples because of their high selectivity, good precision, low cost and simplicity [1]. Sometimes the technique is advantageous over spectrophotometric methods [2]. The development of membrane electrodes of different ionophores including cyclodextrins, calixarenes and other supramolecules of cavity structure which permits guest-host complex formation opened a vast area for ISEs use. The technique is rapidly expanded for determination of different drug substances of various pharmacological groups [3]-[9].

In this paper, three sensors were developed for determination of Fe^{2+} , Mn^{2+} and Zn^{2+} using β -CD, HP- β -CD (Fig. 1) and SCAL (Fig. 2), for sensors 1, 2 and 3, respectively. The ionophores show cone shape with a primary (internal) hydrophobic surface and a secondary (external) hydrophilic surface which permits entrance of different neutral or charged molecules and ions to form inclusion complexes which are based on binding forces being set by several forces as Van der Waals forces and hydrogen bonding [3]. In this work besides the use of macrocyclic ionophores for developing metal sensors, the ability of the cations to be determined, to form complexes with different selective chelating agents was used to increase the selectivity and sensitivity of the developed sensors. The purpose of this manuscript is to determine metal ions in CM of Buffalo-Cows; by a rapid, simple and sensitive method.

Detection of estrus is essential for the efficiency of any breeding program in farm animals and it is the key of success to the insemination management leading to increasing the pregnancy rate and increasing the herd's productivity [10], [11]. The signs of estrus in buffaloes are less noticeable than in cows and the period of postpartum anestrous is usually longer in buffalo than in cattle under comparative management conditions [12]. Silent estrus is the common problem in the buffaloes [13], where detection of estrus behavior is difficult or estrus signs are inconclusive in buffaloes [14]. The CM during estrus shows changes in the metal ions level, physical and chemical properties to facilitate the passage of spermatozoa [15]. There is a very close relationship between the reproductive tract secretions, ovarian

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function and hormonal activity [16]. Researchers found a close relationship between sex hormones in blood and concentration of trace elements in CM at various stages of estrual cycle [17]-[20] beside their importance for proper ovulation process [21]. Also there was significant difference in ionic composition of CM during heat phase and ovulation time in does (female goat) [22]. These factors suggest that an improved management tools especially an automated one, available in the farm for measuring some metallic ions in CM would be valuable. The aims of this work were to develop novel miniaturized sensors for determination of some metallic ions (Fe^{2+} , Mn^{2+} and Zn^{2+}); to be used as sensors for determination of these ions for the purpose of determining the appropriate time for AI; and evaluate the efficiency of the developed simple sensors which offered highly sensitive and selective technique for the determination of ferrous, manganese and zinc ions in CM, by comparison with AAS results.

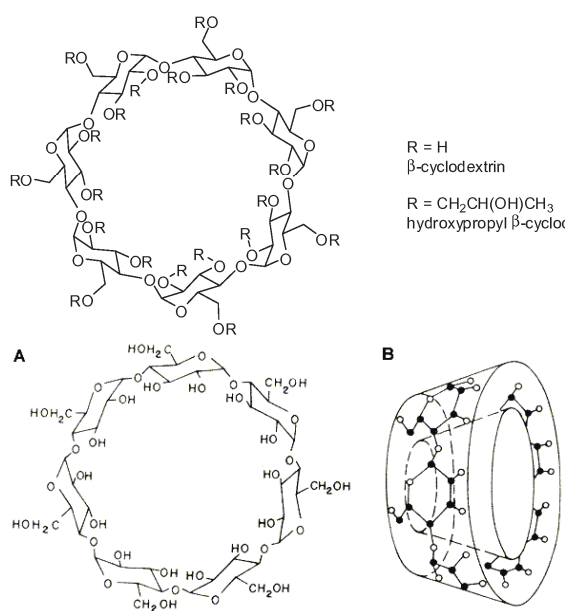


Fig. 1 Structures of β -CD and HP- β -CD [23]

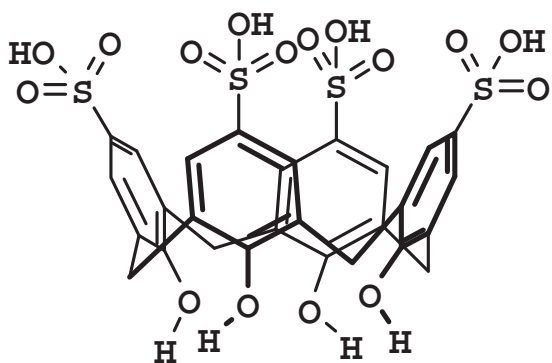


Fig. 2 Structure of SCALSCAL [29]

II. EXPERIMENTAL

A. Measurement of Trace Elements Using Metal Ions Sensors

1. Instrumentation

Jenway, digital ion analyzer, model 3330 (UK), supplied with a reference electrode (double junction Ag/AgCl), model 63178, USA, 314-771-575; Bandolin Sonorox, an Rx510S magnetic stirrer (Hungarian); and a pH glass electrode, Jenway 924008 (UK) are used.

2. Chemicals and Reagents

All chemicals were purchased from Sigma Aldrich (USA); that included, ferrous sulfate heptahydrate, manganese (II) sulfate monohydrate, zinc sulfate heptahydrate, all 99.0%, β -CD, 2-HP- β -CD, SCAL 97%, DOP 99.5%, PVC, bathophenanthroline, porphyrin 90%, oxine 99%, tetrahydrofuran 99.9% anhydrous (THF), potassium chloride 10^{-2} M, sodium hydroxide 0.1 M and 0.1 M hydrochloric aqueous solutions.

3. Standard Solutions

Stock solutions (1×10^{-2} M) of each of ferrous sulfate heptahydrate, manganese (II) sulfate monohydrate and zinc sulfate heptahydrate were prepared by dissolving the appropriate weight of each in 100 mL bi-distilled water; then working aqueous solutions (1×10^{-7} M to 1×10^{-3} M) were prepared by suitable dilution from their stock solutions using bi-distilled water.

4. Procedure

a. Preparation of Electro-Active Coating Membrane

In three separate glass Petri dishes (5 cm), 10 mg of each of bathophenanthroline, porphyrin and oxine were mixed thoroughly with 0.4 mL dioctylphthalate, 0.1 gm PVC and 0.04 gm of β -CD, HP- β -CD and SCAL for sensors 1, 2 and 3, respectively. Each mixture was dissolved in 6 mL THF, and then the Petri dishes were covered with filter paper and left for half an hour to allow slow evaporation of the solvent, producing a thick homogenous coating solution.

b. Fabrication of Coated Graphite Sensors

A rod of graphite (5 mm in diameter and 30 mm in length) was inserted in a polyethylene sleeve and the other end of the protruded rod served as a measuring surface. This end of the rod was washed with acetone, air dried and rapidly dipped into the previously prepared PVC solution. The solvent was allowed to evaporate then re-dipped; evaporation of solvent was done after each dipping. The dipping process was repeated 6 times to produce a uniform membrane on the surface of the graphite rod. The rod was fitted in polyethylene sleeve, the sleeve was filled with equal volumes of 10^{-2} M of potassium chloride and each of 10^{-2} M of Fe^{2+} , Mn^{2+} and Zn^{2+} for sensors 1, 2 and 3, respectively; these solutions were used as internal reference solutions. As an internal reference electrode, Ag/AgCl wire (1 mm diameter) was dipped in the polyethylene sleeve and to ensure electrical contact with the connection cable one drop of mercury was added to the sleeve.

Fig. 3 shows a detailed sketch of a typical fabricated graphite sensor.

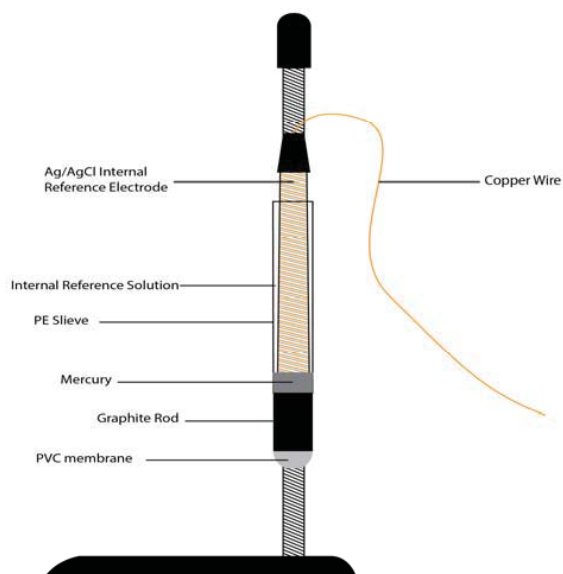


Fig. 3 Detailed sketch of a typical fabricated graphite sensor

The coated graphite electrode was conditioned by soaking in 10^{-2} M ferrous, manganese and zinc solutions for 5 h for sensors 1, 2 and 3, respectively; and stored in the same solution when not in use.

The conditioned sensors were calibrated by separately transferring 50 mL aliquots covering the concentration range 1×10^{-7} M to 1×10^{-2} M metallic ion solution in 100 mL beaker and the potential obtained in conjunction with the reference electrode was recorded. The electrode potential was plotted versus negative the logarithmic concentration of metallic ion solution and the regression equations were calculated and used for subsequent measurements of unknown samples.

5. Effect of pH

The effect of pH on the potential values of the three investigated sensors was studied over pH range of 2-8 at one pH unit interval by using 10^{-4} M ion solutions. The pH was gradually increased or decreased by adding aliquots of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid solutions, respectively.

6. Effect of Foreign Ions

The response of the three developed sensors was examined in the presence of each of the investigated ions. The potentiometric selectivity coefficient ($K^{\text{pot}}_{\text{primary ion, interferent}}$) was used to evaluate the extent to which a foreign ion would interfere with the response of the electrodes to their primary ion.

Selectivity coefficients were calculated by separate solution method (SSM) [24] where potentials were measured for 25 mL of 1×10^{-3} M standard working solution and 1×10^{-3} M interferent solution, separately the potentiometric selectivity

coefficients were then calculated using the rearranged Niclesky-Eisenman equation:

$$\log K^{\text{pot}}_{A,B} = [(E_B - E_A) / (2.303 RT/Z_A F)] + [1 - (Z_A/Z_B)] \log [A]$$

where ' E_A ' is the potential measured in 10^{-3} M standard working cation solution, ' E_B ' the potential measured in 10^{-3} M interferent solution, ' Z_A ' and ' Z_B ' are charges of ion and interferent one, respectively, and $2.303 RT/Z_A F$ represents the slope of the calibration plot (mV/concentration decade).

III. APPLICATION ON CM SAMPLES

A. Animals

This study was carried out at a private farm in Giza (Egypt), where the experimental animals (30 female buffaloes, *Bubalus bubalis*) were multi-parous in 2nd-3rd lactation, weighting 415-530 kg and in good body condition score and maintained under similar conditions. The experimental buffaloes were with 45-65 days postpartum and examined by ultrasound for ovarian activities and genital tract soundness. Nutrient concentration met nutritional requirements according to [25]. The animals are assigned to induced estrus with protocol of synchronization (OVSynch), the animals given on Day 0 (100 µg/mL GONABREED (GnRH)); on day 7 injection of 500 µg per 2 mL of Cloprostenol (PGF2a) and on day 9, 100 µg/mL GONABREED [26], then artificially inseminated on day 10 (16 h after the last injection). The buffaloes were bred and pregnancy diagnosis was performed at 60 days post breeding.

B. Collection of Blood and CM

The buffalo-cows examined rectally before the mucus sample was collected to avoid collecting mucus from buffaloes with cysts or with any abnormal discharge.

Blood and CM were collected from all buffaloes, around heat phase and ovulation, on day 8 of OVSynch protocol, on day 9 (20 h before AI) and on day 10 (1 h before AI). The blood samples were allowed to clot and then centrifuged at 3000 r/min. for 20 min. The sera were collected and stored at -20 °C until assayed for hormone analysis. The mucus samples were collected aseptically by using vaginal scope into a 10 mL glass tube and transported to the laboratory in a refrigerated box (4 °C) for further analysis. The mucus was stored in Eppendorf tubes at -30 °C for determining, the mineral elements (Fe^{2+} , Mn^{2+} , Zn^{2+} and Cu^{2+}). The diluted samples were used for the trace elements estimation using two techniques, atomic absorption according to [27] for the four elements and the developed metal ion sensors for Fe^{2+} , Mn^{2+} and Zn^{2+} .

C. Procedures

1. Determination of Trace Cations Fe^{2+} , Mn^{2+} and Zn^{2+} by the Developed Sensors

The membrane sensors were immersed in conjunction with the reference electrode in each of these samples, and washed with de-ionized water between measurements. The *emf* produced for each sample was measured by the proposed

electrode and then the concentration of the metal ion was determined from the corresponding regression equation.

2. Hormonal Analysis

a. Instrumentation

Microplate Reader EXpERTPLUS, Biochrom Ltd., UK. is used.

b. Progesterone Assay

Progesterone was determined in serum by enzyme immunoassay kit (ELISA), Bio check, Inc. 323 Vintage Park Dr. Foster City, CA 9440412. The absorbance was read at 450 nm within 15 min, a plate of 90well each 1 μ L was used as sample container for microplate reader, according to [21].

3. Measurement of Trace Elements Using Atomic Absorption

a. Instrumentation

The instrument used was Atomic Absorption Spectrophotometer (Mod-3300, Parkin Elmer, USA). Gases used for flame atomizer were acetylene and air; the analytical wavelengths for Cu^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} were 324.8, 248.3, 279.5 and 213.9 nm, respectively. Slits width were 0.7, 0.2, 0.2 and 0.7 nm for Cu^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} , respectively and the relative noise was 1.0 for all.

b. Standard Solutions

Stock standard solutions of copper, iron, manganese, and zinc were purchased from Perkin Elmer in concentrations of 1000 mg/L; being prepared by dissolving 1.0 g of metal in a minimum volume of (1:1) HNO_3 ; then diluted to 1.0 L with 1% (v/v) HCl.

Working standard solutions for the four cations were prepared by suitable dilution using 1% (v/v) HCl in the ranges of 1.0-5.0, 1.5-6.0, 0.5-2.5 and 0.25-1.0 ppm for Cu^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} , respectively.

IV. RESULTS AND DISCUSSION

The CDs structures (Fig. 1) were determined by X-ray crystallography showing that the "truncated cone" has the narrow side formed by the primary hydroxyl groups and the wider side by secondary hydroxyl groups (the cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges). The inclusion of guest molecules into their cavities is one of the most important properties of CDs. This property is based on the presence of the hydroxyl groups of CDs on both inner and outer surfaces which produces a hydrophobic micro heterogeneous environment inside the cavity and a hydrophilic external one which helps to capture suitable hydrophobic guests into this hydrophobic cavity in aqueous media [3]. Calixarenes are ditopic receptor molecules which have wide application in supramolecular chemistry [28]. They are considered as selective ligands for various ions. The phenolic OHs attached on the narrow polar rim of calixarene and the electron rich interior cavities give the suitable environments for formation inclusion complexes through dipole-dipole interactions with metal ion of suitable dimensions, as shown in

Fig. 2. They have the ability to form complexes with considerable number of cations in the form of host-guest inclusion complexes. An excellent review was given by [29]. Depending on this property, a number cations selective electrodes were developed [30]-[32].

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline), Oxine (8-hydroxyquinoline) and Porphyrin (H_2P) are excellent chelating agents for metal ions; H_2P and its derivative [H_2Ps] form complexes with square-pyramidal structure [33]. Their use in analytical chemistry had been reviewed by [33]. Also, the use of [H_2Ps] for analysis of trace elements by HPLC was demonstrated by [34]. H_2P as an ionophore in PVC membrane using different plasticizers and sodium tetra phenyl borate (NaTPB) as anion excluder was developed as an ISE for Zn^{2+} ion [35] Zinc ISEs based on inclusion complexes formation with different macromolecules ionophores other than CDs were previously mentioned [36]-[38].

In this work, three sensors were developed using three ionophores, namely β -CD, HP- β -CD and SCAL for trace determination of Fe^{2+} , Mn^{2+} and Zn^{2+} using DOP plasticizer.

The three positive cations to be determined in this study have high preference to donation sites OH groups and sulphonic acid of β -CD, HP- β -CD and SCAL and calixarene structures, respectively, which encourage their use as ionophores for preparation of selective membranes. β -CD was chosen for Fe^{2+} (sensor 1), HP- β -CD for Mn^{2+} (sensor 2), while for Zn^{2+} , SCAL was used (sensor 3). Upon determining the sensitivity and selectivity of the sensors towards cations, poor results were obtained. This is may be due to the fact that the three transition metals ions have close radii (≈ 0.6 nm); which could result in poor preference to certain ion over the others upon forming guest host complexes with the ionophores. Thus, to improve the sensors performance, metal chelating agents were incorporated with PVC-ionophore membranes; for sensor 1 used for Fe^{2+} , bathophenanthroline was the chelating agent, while porphyrin and oxine were that used for Mn^{2+} and Zn^{2+} , respectively. The introduction of such chelating agents in the membrane fabric would selectively enrich the host cavity of the ionophore with high charge density from the complexed ion, leading to increase in both sensitivity and selectivity. An illustration of the electrode design is given in Fig. 3.

Evaluation of the Electrochemical Performance Characteristics

Electrochemical performance characteristics of the presented sensors according to the IUPAC guidelines [24] were presented in Table I.

Calibration plots of the three sensors are shown in Fig. 4. The slopes of the calibration plots approached double Nernstian electrode slope by the proposed ionophore-based membranes as observed for the Fe^{2+} 53.46, Mn^{2+} 45.01 and Zn^{2+} 50.96; this phenomenon was explained on the basis of equilibrium processes occurring at the sample-membrane interface, a twice Nernstian response may arise from the inverse relationship between metal ion activity in the aqueous phase *al* (*aq*) and the uncomplexed ion activity in the organic

phase boundary aI (org) [39]. Also deviation from the ideal Nernstian response slope (60 mV) stems from the fact that the electrodes respond to the activities of cations rather than its concentration. These responses appear if aI (org) varies linearly with aI (aq) where aI [40]. The potential displayed by the proposed electrodes upon measuring standard drug solutions with concentrations within the linearity range in the same day or from day-to-day did not vary by more than ± 1 mV. Calibration slopes did not change by more than ± 1 mV/decade concentration over a period 8, 10 and 21 days for sensors 1, 2 and 3, respectively. The required time for the electrodes to reach values within ± 1 mV of the final equilibrium potential after increasing the ion concentration 10-folds was found to be 10 sec (dynamic response).

TABLE I
POTENTIOMETRIC RESPONSE CHARACTERISTICS OF THE THREE INVESTIGATED SENSORS

Parameter	Fe ²⁺ (Sensor 1)	Mn ²⁺ (Sensor 2)	Zn ²⁺ (Sensor 3)
Slope (mV/decade)	53.46	45.01	50.96
Intercept (mV)	336.0	521.0	1,210.0
Concentration range (M)	10 ⁻² - 10 ⁻⁷	10 ⁻² - 10 ⁻⁷	10 ⁻² - 10 ⁻⁷
LOD* (M)	0.8 \times 10 ⁻⁷	0.6 \times 10 ⁻⁷	0.2 \times 10 ⁻⁷
Working pH range	4-8	4-8	4-8
Response time (Sec.)	10.0	10.0	10.0
Stability (Days)	8.0	10.0	21.0
Recovery \pm SD%**	98.93 \pm 1.01	96.54 \pm 2.01	99.94 \pm 0.85
Correlation Coefficient	0.9996	0.9995	1.0000

* Limit of detection (measured by the interception of the extrapolated arms of Fig. 4).

** Average of five determinations.

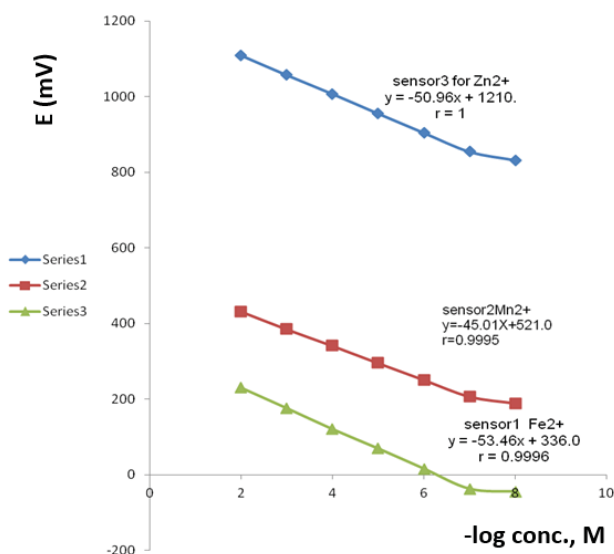


Fig. 4 Profiles of the potential (in mV) vs. -log concentrations of metal ions sensors 1, 2 and 3

Effect of pH

The potential pH profile obtained indicates that the responses of the three sensors were fairly constant over the pH range 2-8. Above pH 8, precipitation of ions took place as hydroxide and sudden drop in potential occurs as observed

from Fig. 5. Thus, the range of 4-8 was used where the sensor response is fairly stable.

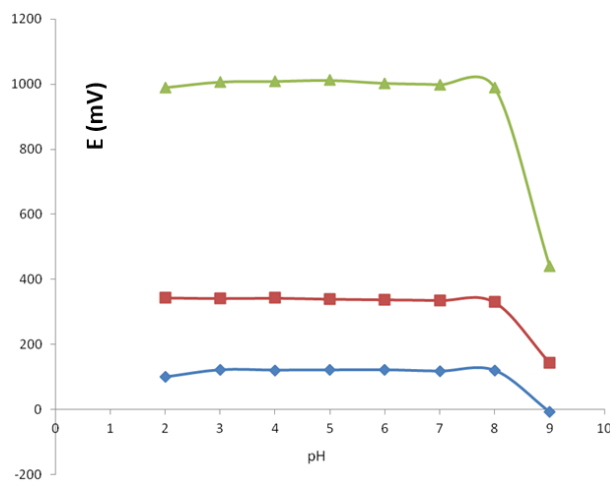


Fig. 5 Effect of pH on the response of sensors 1, 2 and 3 using 10⁻⁴ M

Sensors Selectivity

To evaluate the selectivity of each sensor towards the other investigated cations, the selectivity coefficient was calculated using Nicolsky-Eisenman equation [24]. The results are given in Table II, which reveal that the proposed membrane sensors display high and equivalent selectivity to each selective ion membrane regarding the other significant ions in the targeted samples.

TABLE II
POTENTIOMETRIC SELECTIVITY COEFFICIENTS FOR THE THREE PROPOSED SENSORS

Interferent Ion	Sensor 1 (for Fe ²⁺)	Sensor 2 (for Mn ²⁺)	Sensor 3 (for Zn ²⁺)
Fe ²⁺	Zero	4.8 \times 10 ⁻³ M	2.6 \times 10 ⁻³ M
Mn ²⁺	5.1 \times 10 ⁻³ M	Zero	3.7 \times 10 ⁻³ M
Zn ²⁺	5.5 \times 10 ⁻³ M	8.2 \times 10 ⁻³ M	Zero
Cu ²⁺	2.8 \times 10 ⁻³ M	7.2 \times 10 ⁻³ M	6.1 \times 10 ⁻³ M

Application for Determination of Fe²⁺, Mn²⁺ and Zn²⁺ in CM of Egyptian River Buffalo Cows (*Bubalus bubalis*)

The results obtained by measuring Fe²⁺, Mn²⁺ and Zn²⁺ for several samples during applying induced estrus with protocol of synchronization (OVSynch) program; on days 8, 9 (20 h before AI) and on day 10 (1 h before AI) using the newly developed sensors and those obtained by AAS method are presented in Table III.

Statistical comparisons between the results obtained by the two methods for samples taken on day 8 of the synchronization program are shown in Table IV. The statistical analysis showed that there is no significant difference for analyzing Fe²⁺ by the proposed sensor 1 and AAS as expressed by t- and F-values which are lower than the critical values. On the other hand, in case of Mn²⁺ and Zn²⁺, the two methods appeared to be at the same level of accuracy (t-student test with t-value lower than the critical value) but there was a significant difference with better precision

performance of the potentiometric method (lower SD and variance values). The F-ratio was higher than the critical theoretical value.

TABLE III
RESULTS OF DETERMINATION OF Fe^{2+} , Mn^{2+} AND Zn^{2+} IN CM SAMPLES BY SENSORS (B) AND ATOMIC ABSORPTION SPECTROPHOTOMETRY (A)

Ion to be determined Sample No.	Fe^{2+} (Sensor 1)		Mn^{2+} (Sensor 2)		Zn^{2+} (Sensor 3)	
	A* ppm	B** ppm	A*	B**	A*	B**
1 ^a	2.43	2.33	0.50	0.56	3.78	3.80
2 ^a	2.50	2.34	0.57	0.55	4.50	3.80
3 ^a	2.02	2.11	0.55	0.55	3.74	3.70
4 ^b	2.10	2.14	0.46	0.47	1.60	1.60
5 ^b	2.10	2.14	0.48	0.49	1.42	1.50
6 ^b	2.80	2.57	0.49	0.51	1.80	1.60
7 ^c	2.88	2.93	0.48	0.47	1.25	1.40
8 ^c	2.77	2.84	0.47	0.47	1.17	1.10
9 ^c	2.80	2.55	0.45	0.47	1.40	1.40

* AAS results [mg/L (part/million)].

** Potentiometric results [mg/L (part/million)].

^a On day 8 of the synchronization program.

^b On day 9 (20 h before AI).

^c On day 10 (one hour before AI).

All results are the mean of three determinations.

TABLE IV
STATISTICAL COMPARISON BETWEEN THE RESULTS OBTAINED BY THE SENSORS AND AAS METHODS

Ion to be determined The Method	Fe^{2+} (Sensor 1)		Mn^{2+} (Sensor 2)		Zn^{2+} (Sensor 3)	
	A* ppm	B** ppm	A* ppm	B** ppm	A* ppm	B** ppm
Mean	2.32	2.26	0.54	0.55	4.00	3.76
N	3	3	3	3	3	3
SD	0.259	0.130	0.036	0.005	0.428	0.057
Variance	0.0672	0.0169	0.0013	0.000032	0.1830	0.0033
F	3.978 (19) ¹		39.000 (19) ¹		54.800 (19) ¹	
t	0.757 (3.182) ²		-0.632 (4.302) ²		0.963 (4.302) ²	

* AAS results [mg/L (part/million)].

** Potentiometric results [mg/L (part/million)].

¹ The value between parenthesis are critical values of F at P=0.05.

² The value between parenthesis are critical values of t at P=0.05.

The correlation between the elements concentrations in CM and serum progesterone hormone P_4 was performed by carrying out statistical analysis. The results were expressed as mean \pm standard error (SE). ANOVA one way test was used to compare progesterone, cervical elements concentrations and electrical conductivity in three times, on days 9, just before administration of GnRH injection, 18 h and 2 h before AI using SPSS program. All analyses were performed according to [41]. The correlation is presented in Table V.

TABLE V
CORRELATION BETWEEN SOME CERVICAL MICROELEMENTS AND SERUM PROGESTERONE HORMONE (P_4)

Cervical micro-elements	Correlation with P_4 (r)	F	T	P-value
Fe^{2+}	-0.376	9.440	4.895	0.000008*
Mn^{2+}	+0.409	11.457	-2.866	0.00584*
Zn^{2+}	+0.893	225.208	-1.782	0.07800

* Significantly different at $P > 0.01$.

V. CONCLUSION

Three miniaturized PVC sensors on solid state graphite support were developed, for electrochemical evaluation of ferrous, manganese and zinc ions in CM for, determining the appropriate time for AI of Egyptian river buffalo cows. To increase the sensitivity and selectivity of the sensors, appropriate chelating agent for each were incorporated in the membrane. The simplicity, high selectivity and sensitivity of the method together with low cost and short time of preparation of the sensors favour their use.

Examining the trace elements concentration in CM showed that Zn^{2+} decreases significantly at the period of ovulation this result was correlated with progesterone hormonal analysis and ultrasound clinical examinations which in-conclusion suggest significant differences and powerful correlation of progesterone with Zn^{2+} composition of CM during heat phase and the ovulation time.

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