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Determination of zinc in infertility men serum and semen plasma using Square Wave Voltammetric and Atomic Absorption Methods

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Abstract: The electrochemical behavior of Zn-acetate. $2H_2O$ was carried out using square wave voltammetric method (S.W.V) at HMDE. A well defined peak was observed at (-0.9864) volt versus the reference electrode (Ag/AgCl, Sat KCl). The calibration curve of zinc with oxidation number (II) in the phosphate buffer (pH=7.0) has been studied. The relationship was linear within the scope of concentration (2.172×10^{-2} - 47.190×10^{-2}) ppm. The correlation coefficient is (R = 0.9985). Another two calibration curves were also constructed in the presence of fertile serum and semen plasma in men. The study showed that zinc can be determination by the two methods (S.W.V and Atomic absorption) and the same accuracy, also showed there is no significant difference in the concentration of serum zinc in the infertile male compared with the control group, as well as showed a low significant difference in the concentration of zinc at (P = 0.05) in the semen of infertile male compared with the control group.

Keywords: Zinc, Infertility men, Genetic problems.

INTRODUCTION

Infertility is defined by most authorities as the inability to achieve pregnancy after one year of unprotected intercourses[(1)]. A large proportion of infertile men fail to impregnate their female counterpart because of lack of sperm (azoospermia) or too little sperm (oligozoo spermia)[(2)]. The clinical evaluation of male infertility includes a detailed history, physical examination, laboratory tests, ultrasound study and karyotyping. The two main purposes of the evaluation to identify any modifiable factors that can improve the man's fertility status and to identify any serious underlying conditions, such as testis cancer, osteoporosis and endocrine or genetic problems that present first as infertility[(3)]. In fact elements calcium, magnesium, copper, selenium and zinc play very vital role in effecting various parameters of semen. Among trace elements increasing evidence of a direct relationship of zinc was found with seminal parameters[(4)]. Zinc is a micronutrient abundantly present in meat and sea food. It is the second most abundant trace element the body totaling nearly 2 gm, essential for normal functioning of the male reproductive system, numerous biochemical mechanisms are zinc dependant, including more than 200 enzymes in the body. Zinc is a natural aromataze enzyme inhibitor. Aromataze enzymes cause the body to block the pituitary gland from releasing lutein and follicle stimulation of hormones which stimulate the production of testosterone, aromataze enzyme converts testosterone into estrogen and result in lower amounts of available testosterone. Zinc is not only vital in the production of testosterone, it also works to maintain healthy semen volume and has been implicated in testicular development and sperm maturation[(5)]. Several methods used to determine zinc, the adsorptive collection of zinc complex with alizarin ligand, coupled with the square wave voltammetric technique at the HMDE.

The monitored stripping voltammetric current was linear over the range of (5×10^{-8} - 4×10^{-7} $moll^{-1}$) and the detection limit was (1×10^{-8} $moll^{-1}$)[(6)]. The conditions of voltammetric determination of zinc compound pharmaceutical preparations were established and validated. The concentration of zinc in solution was determined by differential pulse voltammetry (DPV). Zinc was determined within the concentration range of (1-12) ppm[(7)]. Zinc determination in Zn-Fe alloy galvanic baths was developed employing square wave voltammetry with the static mercury drop electrode (SMDE) as working electrode. The proposed voltammetric method showed a linear response range between (1.0×10^{-5} - 2.2×10^{-4} $moll^{-1}$) for zinc[(8)]. The reverse phase HPLC method was developed for the determination of zinc carnosine in pharmaceutical dosage forms. A linear responses in the concentration range of (2-10 µg/ml) of zinc carnosine[(9)]. The present work involves the use of square wave voltammetric behavior of Zn-acetate. $2H_2O$ for a direct determination of zinc in infertility men serum and semen plasma and compared with atomic absorption technique.

EXPERIMENTAL

Apparatus:

Voltammetric measurements were carried out using a Metrohm instrument, model 797 VA, computrace with stand three-electrodes containing a HMDE as a working electrode . Ag/AgCl ,Sat. KCl as reference electrode and a platinum wire as an auxiliary electrode.The pH of the solutions was controlled with a HANA pH meter. Atomic absorption spectrophotometer from GBC scientific equipment, Australia model 2010.

Reagents:

All the chemicals used were of analytical reagents grade. The supporting electrolyte used for all experiments was phosphate buffer which was prepared by mixing certain amounts of 0.2 M of each of K_2HPO_4 and KH_2PO_4.

Zn-acetate. $2H_2O$ (2.19×10^2) ppm:

was obtained from Aldrech prepared freshly by dissolving (0.00109 gm) Zn-acetate. $2H_2O$ in deoionised water absolute and the solution was diluted to 5ml with deoionised water in a (5 ml) volumetric flask. The solution was kept in a refrigerator at 4 ^{0}c in dark.

Samples collection:

The study included collection of samples attending the Hospitals in Mosul, from 80 men divided to two groups (30 healthy men's and 50 men diagnosed with infertility only).

Procedure:

The square wave voltammetric mode was used with deposition time 50 sec; Frequency 50 Hz ; equilibration time 55 sec ; Voltage step 0.006 V ; Amplitude 0.02 V. The solution was de-aerated by passing through it a slow stream of purified nitrogen gas for 10 minutes to remove the dissolved oxygen. The square wave voltamogram was recorded on a degassed phosphate buffer solution at pH=7.0 (10 ml). The back current was recorded, appropriate amount of (1×10^{-4}) M Zn-acetate. $2H_2O$ stock solution were added to this solution to yield the desired concentration and the current – voltage current was recorded again. The calibration curve was then constructed. Another two calibration curves were also constructed using the same procedure above in the presence of (10 µl) fertile serum (ten times diluted) and (10 µl) semen plasma.

Atomic absorption method:
Preparation of samples:
Serum and semen plasma diluted (5-10) times with deoinized water.

Preparation of standard curve for zinc:

To prepare the standard zinc solution concentration (1000 µg/ml), were thawed (0.1 gm) of zinc element in (3 ml) of (5.0) M hydrochloric acid diluted by HCl (1:1) with deoinized water, and transfer the solution to a volumetric bottle (100 ml), and completed the volume with deoinized water, and then standard solutions of different concentrations (0.5 – 2.5 µg/ml) were prepared, absorbance was measured at 213.9 nm as shown in fig.1

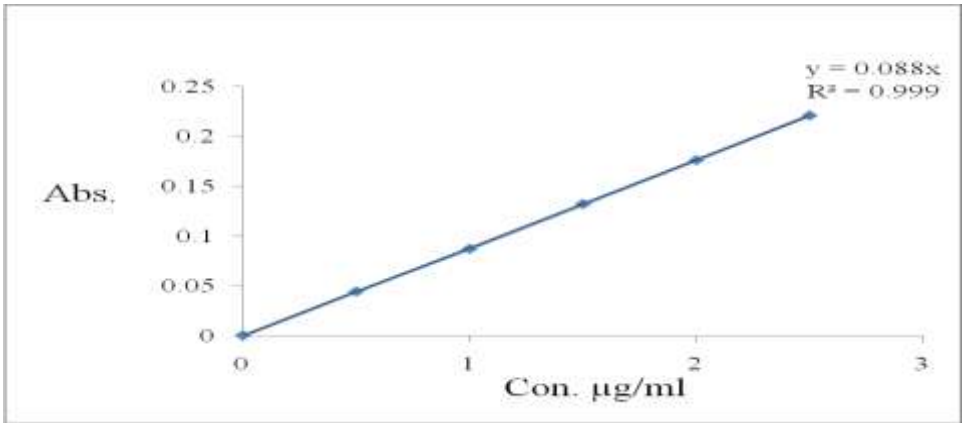

Fig.(1): Standard curve of zinc by Atomic absorption method of (0.5 – 2.5 µg/ml).

Statistical method:

The statistical methods used to analysis the data include mean, standard deviation ,minimum and maximum, while T-test and Denken -test was used to compare between total control and patients[10].

RESULT AND DISCUSSION

Typical square wave voltamogram of (21.92 $x10^{-2}$) ppm Zn-acetate.$2H_2O$ in phosphate buffer at (pH=7.0) as shown in fig. 2

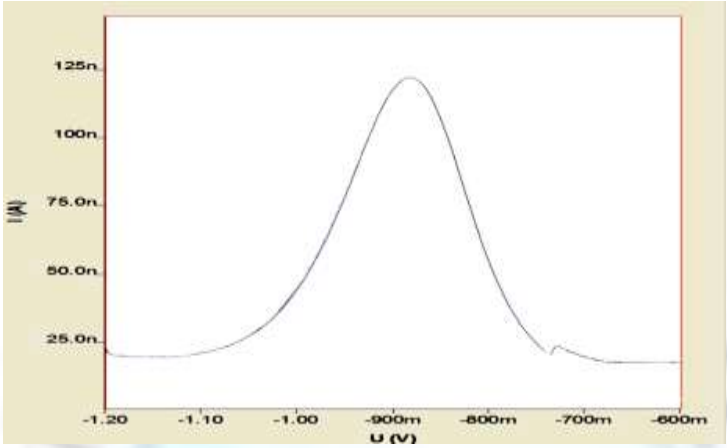

Fig. (2) : Square wave voltamogram of (21.92 $x10^{-2}$) ppm Zn-acetate.$2H_2O$ in phosphate buffer at (pH=7.0).

It can be seen from fig.2 a well-defined peak appeared at (-0.9864 V) versus (Ag/AgCl,Sat KCl) electrode.
Optimum condition: The square wave voltammogram of (6.56×10^{-2}) ppm Zn-acetate. $2H_2O$ was investigated in phosphate buffer (pH=7.0) variation all the parameters that it depend on the measurement in Table (1).

Table (1) : Show the optimum values obtained which give either the highest peak current and the best resolution of the peak

Condition	Value	Condition	Value
initial pot.	- 1.20 V	Frequency	50 Hz
final pot.	- 0.6 V	Voltage step	0.006 V
deposition time	50 second	Amplitude	0.02 V
Equilibration time	55 second		

Effect of pH:

The square wave voltamogram of (2.17) ppm of Zn-acetate. $2H_2O$ were investigated at different pH values (6-9) using the optimum condition in phosphate buffer show in Table (1).The peak current (Ip) and peak potential (Ep) obtained are shown in Table (2) .

Table (2) : Effect of pH on S.W.V.peak and peak current of (2.17) ppm of Zn-acetate. $2H_2O$

pH	Ep (v)	Ip (nA)
6	- 0.993	174
7	- 0.999	528
8	- 0.993	404
9	- 0.999	234

The peak current (Ip) is clearly dependent the pH. Maximum current response was found at (pH=7.0).On the other hand the peak potential (Ep) is found to be independent on pH .

Stability of Zn-acetate. $2H_2O$ in aqueous Phosphate buffer:
The square wave voltamogram of (43.67×10^{-2}) ppm of Zn-acetate. $2H_2O$ were recorded at different times in phosphate buffer at (pH = 7.0). The result obtained are tabulated in Table (3) .

Table (3) : Effect of time on SWV peak of ($43.67x10^{-2}$) ppm of Zn-acetate. $2H_2O$ at (pH=7.0) in aqueous solution.

Time (min)	Ip (nA)
3	67.4
6	67.4
9	65.7
12	66.2
15	65.8
18	64.1
21	65.7
24	63.7
27	64.6
30	65.5
33	67.6
36	65.9
39	66.1
42	66.8
45	64.8

It can be seen from the Table (3) that Zn-acetate. $2H_2O$ is stable for more than 45 minute.

Analytical Consideration:

Using the optimum condition showing in Table (1), the calibration curve was constructed using a serial dilution of a standard Zn-acetate. $2H_2O$ in aqueous-phosphate buffer (pH=7.0) (10 ml). Some typical results are listed in Table (4). These solutions were prepared by adding appropriate aliquots of standard Zn-acetate. $2H_2O$ to the phosphate buffer (10 ml) at (pH=7.0) .

Table (4) : Effect of concentration on peak current of (2.17×10^{-2} - 47.19×10^{-2}) ppm of Zn-acetate. $2H_2O$ at (pH=7.0) in aqueous solution at Ep = -0.9864 V.

Conc. (ppm) 10^{-2}	Ip (nA)
2.17	19.6
4.36	22.7
6.56	24.5
8.73	27.5
10.90	30.4
13.08	33.2
15.25	35.8
17.40	39.1
19.55	41.3
21.72	43.6
26.11	49.3
30.28	53.7
34.45	58.4
38.63	63.6
43.02	66.5
47.19	70.8
R	0.9985
R^2	0.9971
Slope	1.1579
Intercept	17.939

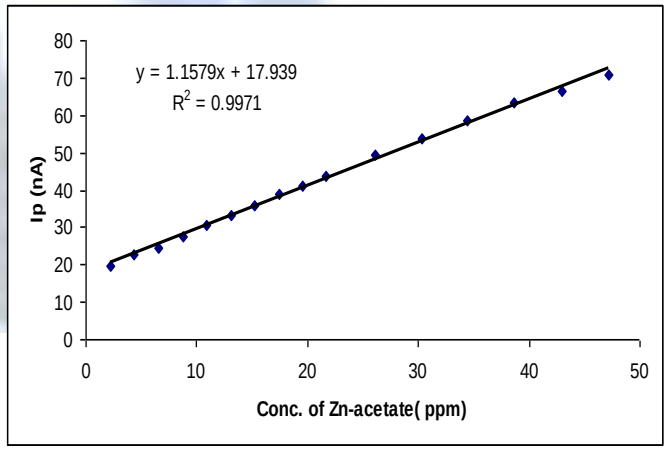

Fig. (3):The relation between peak current (Ip) and concentration of (2.17×10^{-2} – 47.19×10^{-2}) ppm of Zn-acetate. $2H_2O$ at (pH=7.0) in aqueous solution at Ep = -0.9864 V.

The plot peak current Ip versus molar concentration of Zn-acetate. $2H_2O$ are showing in fig. 3. Regression analysis on standard indicated straight linear on the concentration range of (2.17×10^{-2} – 47.19×10^{-2}) ppm with correlation coefficient (R=0.9985). The lowest experimental detection limit was (2.17×10^{-2}) ppm.

Effect of Concentration (Calibration Curve of Zn-acetate. $2H_2O$) with Fertile men Serum

Using the optimum condition showing in Table (1), the calibration curves were constructed using a serial dilution of a standard Zn-acetate. $2H_2O$ in human serum. Some typical results are listed in Table (5). These solutions were prepared by adding appropriate aliquots of standard Zn-acetate. $2H_2O$ to the phosphate buffer (10 ml) at (pH=7.0), containing (10 µl) fertile serum. Only one peak for Zn-acetate. $2H_2O$ was observed at (E_p = - 0.855 V).

Table (5) : Effect of concentration on peak current of (2.19 – 21.70) ppm of Zn-acetate. $2H_2O$ in the presence of fertile men serum (pH = 7.0) at (Ep = - 0.855 V).

Conc. (ppm)	Ip (nA)
2.19	63.7
4.36	130
6.56	207
8.73	272
10.90	331
13.08	408
15.23	443
17.40	494
19.55	541
21.70	585
R	0.9953
R2	0.9908
Slope	26.82
Intercept	26.434

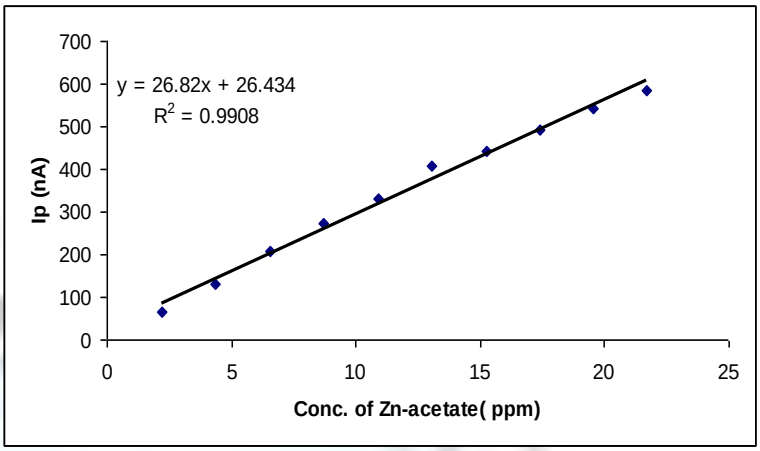

Fig. (4) : the relation between peak current (Ip) and concentration of (2.19 – 21.70) ppm of Zn-acetate. $2H_2O$ at (pH=7.0) with human serum at Ep = -0.855 V.

$2H_2O$ are showing in fig. 4. Regression analysis on standard indicated straight linear on the concentration range of (2.19–21.70) ppm with correlation coefficient (R = 0.9953). The lowest experimental detection limit was (2.19) ppm.

Effect of Concentration (Calibration Curve of Zn-acetate. $2H_2O$) with Fertile Semen Plasma.
Using the optimum condition showing in Table1, the calibration curves was constructed using a serial dilution of a standard Zn-acetate. $2H_2O$ in fertile semen plasma. Some typical results are listed in Table (6).These solutions were prepared by adding appropriate aliquots of standard Zn-acetate. $2H_2O$ to the phosphate buffer (10 ml) at (pH=7.0), containing (10 µl) fertile semen plasma. Only one peak for Zn-acetate. $2H_2O$ was observed at (E_p= - 0.891 V).

Table (6) : Effect of concentration on peak current of (0.21 - 4.30) ppm of Zn-acetate. $2H_2O$ in the presence of fertile semen plasma (pH = 7.0) at (Ep = - 0.891 V) .

Conc. (ppm)	Ip (nA)
0.21	63
0.43	76
0.65	89
0.87	101
1.09	121
1.30	138
1.52	151
1.73	158
1.95	181
2.17	195
2.58	227
3.02	250
3.44	279
3.88	302
4.30	327
R	0.9989
R2	0.9979
Slope	66.083
Intercept	48.824

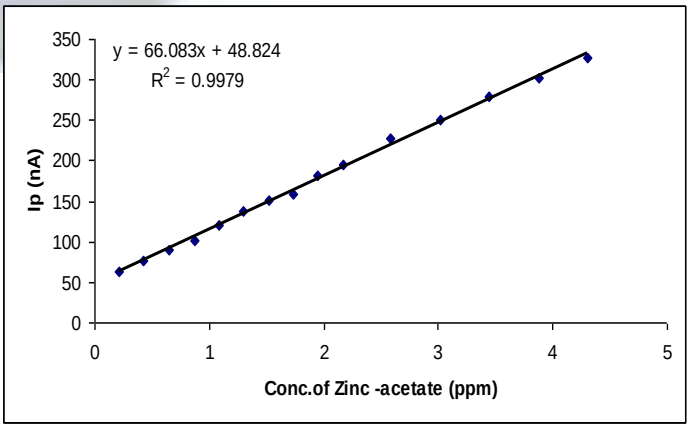

Fig. (5) : the relation between peak current (Ip) and concentration of (0.21 - 4.30) ppm of Zn-acetate. $2H_2O$ at (pH=7.0) with fertile semen plasma at Ep = -0.891 V.

The plot peak current Ip versus molar concentration of Zn-acetate. $2H_2O$ are showing in fig. 5. Regression analysis on standard indicated straight linear on the concentration range of (0.21-4.30) ppm with correlation coefficient (R= 0.9989). The lowest experimental detection limit was (0.21) ppm.

Applications of proposed method

Determination of Zn element in human serum and semen plasma:

Results are expressed as mean ± S.D for each parameter, statistically significant differences among oligospermic & normospermic control groups are indicated along with their significant values. The method was successfully applied for determining zinc in fertile serum and semen plasma by S.W.V. and Atomic absorption methods. Using the optimum condition in Table (1). A standard addition method was used to calculate the concentration of zinc in fertile serum and semen plasma, the zinc concentration in infertile male serum and semen plasma in the four groups are shown in Table (7).

Table (7) : The results of Zn in fertile serum and fertile semen plasma.

S.W. Voltammetric method				Atomic absorption method			
Parameter	Patients n=50 Mean ± S.D	Controls n=30 Mean ± S.D	P-value %	Parameter	Patients n=50 Mean ± S.D	Controls n=30 Mean ± S.D	P-value %
Zinc in human serum µg/ml	c 0.114 ± 0.07	bc 0.228 ± 0.05	N.S	Zinc in human serum µg/ml	c 0.118 ± 0.06	bc 0.198 ± 0.08	N.S
Zinc in semen plasma µg/ml	*bc 0.220 ± 0.01	bc 0.337 ± 0.11	0.05	Zinc in semen plasma µg/ml	*a 0.224 ± 0.02	ab 0.387 ± 0.13	0.05

* P-value significant difference at (P = 0.05).

The results in table 7 showed a low concentration of serum zinc in the infertile male compared with the control group but it's not significant difference, as well as showed a low significant difference in the concentration of zinc at (P = 0.05) in the semen of infertile male compared with control group this may be due to the role of zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin[(11)], also zinc deficiency is associated with decrease testosterone concentration and sperm count[(12)]. Finally the study showed no significant difference in concentration of zinc in serum and semen using S.W.Voltammetric method compared with atomic absorption method.

Conclusion

A very simple direct voltammetric and atomic absorption methods were reported for determination of zinc in infertility men serum and semen plasma, zinc may contribute to fertility through its significant effects on various serum and semen concentration. It seems that the determination of serum and semen zinc may help in investigation and treatment of infertile male. The two methods have same accuracy to determination of zinc in serum and semen.

References

[1]. Olayemi F. O., African Journal of Biotechnology , Vol. 9 (20) , pp: 2834-2842 , 17 May , 2010 .
[2]. Opoku A. et al , British Journal of Medicine and Medical Research, 4 (6) : 1432-1441 , 2014.
[3]. Lipshultz L.I. , L.I. Howards , and C. S. Niederberger , Infertility in the Male , Cambridge university press , Cambridge, uk , 4th edition , 2009 .
[4]. Barbara B. , Men contribute to and suffer from Infertility , Family Health International , 23 ; 17-20, 2003.
[5]. Steven Sinclair . Male infertility – nutritional and environmental considerations. Alternative Medicine Review, 5(1) :28-38; 2000.
[6]. Ahmad H. , Alghamdi ; Journal of Saudi chmical society, volume 14 , issue 1, January, pages : 1-7, 2010.
[7]. ANNA LUT KA and HONORATA BUKOWSKA ; Acta Poloniac Pharmacentica-Drug Reseaech . vol. 66 No. 5, pp. 471-475 , 2009 .
[8]. Negiane Favaron and M. Luiz ; J. Braz . chem. . soc. Vol. 12 no.2 sãopaulo Marr,Apr. 2001.
[9]. Panjagala satyanarayana et al, International Journal of chem., Tech. Research CODEN (USA) , vol. 4 , No. 2 , pp.842-846 , April-June , 2012.
[10]. Kirkpatrick,L. and B.C.Feeney,.A simple guide to IBM spss statistics for versions 18.0 and 19.0.11th Edn.,Wadsworth cengage learning, Belmont, ISBN-10: 1111352550 ,pp:115. 2012
[11]. SRS-Stanwell, A. Thompson, R. Haines. A comparative study of zinc., copper, cadmium and lead levels in fertile and infertile men. Fertil steril ; 40 ; 670-70; 1983.
[12]. Masayuki Kaji. zinc in endocrinology, International Pediatrics ; 16(3) : 3-7; 2001.