Determination of Zinc Trace Element in Rheumatoid Arthritis Patients in Some Areas of Saudi Arabia Kingdom by Anodic Stripping Voltammetry Technique

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Abstract: Due to the importance of trace elements, Zinc (Zn) trace element have been determined in blood, serum, plasma, plasma clot and serum clot samples by using stripping voltammetry techniques for both healthy and patients with rheumatoid arthritis (R.A) in Jizan and Jeddah cities. Statistical analysis was made to analyze the results by use Friedman test at significant (P>0.01) in the different studied samples. Factors that effect on stripping voltammetry of Zinc element in standard solutions, healthy and rheumatoid arthritis patients samples in Jeddah and Jizan cities in different studied samples have been studied (deposition time, deposition potential, pulse time, pulse amplitude, voltage step, time voltage step time, drop size, Equilibration time) on each of voltage and current of behavior of electrical for zinc element under study to identify the best conditions and factors that effect on determination of concentration Zn element using this technique.

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Keywords: Rheumatoid Arthritis; Saudi; voltammetry; Zn trace element.

1. Introduction.

Zinc (Zn) is an essential trace element required for normal function of multiple enzymes, hormones, and transcription-related factors [1,2]. Zn homeostasis is required for normal functioning of the immune system, insulin secretion/action, and anti-oxidant systems, all processes known to be disturbed in critical illness. Pediatric and adult studies have consistently demonstrated abnormally low zinc levels in critically ill patients as inflammation and infection are associated with reduced serum levels of zinc[3-8]. Zn supplementation may be a beneficial therapeutic strategy in critically ill patients [9-12]. However, for this strategy to work safely, serum zinc levels must be monitored constantly. The conventional methods for measuring Zn in bodily fluids are accurate, but costly and time consuming. For example, Rahman *et al.* [13] used instrumental neutron activation analysis (INAA) and atomic absorption spectrophotometry (AAS) to validate quantification of Zn in whole blood of cardiovascular diseases (CVD) and malignant hypertension (MH) patients. Barany *et al.* [14] used inductively coupled plasma mass spectroscopy (ICP-MS) to measure multiple trace elements including Zn in blood and serum of adolescents. The most critical challenge of these conventional methods is the time delay from sample collection, shipment to a certified metals laboratory, and reporting results to a clinician. Also, laboratory costs associated with these repeated measurements may be high. These challenges are particularly important when dealing with critically ill patients. Stripping analysis is a powerful yet simple approach to measure trace metal concentrations. It offers very low limits of detection and simple instrumentation. Thus, it is preferred for "on-site" applications and can be miniaturized for portable and inexpensive field operations [15,16]. Mercury electrodes are typically used in stripping analysis and produce reliable measurements. Different forms of mercury electrodes have been reported in literature for the detection of Zn including hanging mercury drop electrode (HDME), mercury thin film electrode (MFE), chemically modified mercury film electrode and mercury film on screen printed carbon-paste electrodes [17-22]. However, the toxicity of mercury electrodes leads to strict safety and handling requirements, presenting a critical challenge for "on-site" applications. This search study the behavior and factors that effect on stripping voltammetry of Zinc element (deposition time, deposition potential, pulse time, pulse amplitude, voltage step, time voltage step time, drop size, Equilibration time) on each of voltage and current of behaviour of electrical zinc element under study to identify the best conditions and factors that effect on determination of concentration Zn element using this techniquein standard solution and all samples extracted (blood, plasma, serum, clot serum, clot plasma) of healthy and patient rheumatoid arthritis(R.A)in Jeddah and Jizan cities

2. Material and Method:

Samples Collection:

47 blood samples were collected (7 samples from healthy, 20 samples from R.A. patients in Jeddah area and 20samples from R.A. patients in Jizan cities).

Preparation of the samples:

1 ml from the sample is treated with 5 ml concentrated HNO_3acid in the digestion vessel. Steaming the solution is made on hotplate until the volume of solution is reduced to 1 ml. Then ml of double distilled water is added. This addition of water is repeat many times to get rid of the extra HNO_3acid.

The devices used in the study:

Trace elements were measured by Polarograph instrumental 746VA trace analyzer with 747VA stand from Metrohm Company. The information storage is done by a computer, from Toshiba Company 757 VA joined with the device.

3. Results and discussion:

Table (1) and figure (1): show concentrations of Zn element in the samples (blood, plasma, serum, clot serum, plasma clot) for Healthy, R.A. Jizan, and R.A. Jeddah cities using Friedman test at significant ($P > 0.01$).The study showed that Zn for healthy samples in all the studied tests was higher, also, the study shows that the lowest concentration of element zinc in plasma is (0.2314 ppm), while highest concentration of element zinc in clot of serum is (0.5014 ppm).

Table (1): Concentration of Zinc Element in Healthy, R.A. Jizan, and R.A. Jeddah cities.

Test	Mean Conc. (ppm) ± S.D		
	Healthy	R.A. Jizan	R.A. Jeddah
Blood	0.4042 ± 0.0179	0.1895 ± 0.0632	0.1610 ± 0.0172
Plasma	0.2314 ± 0.0229	0.1245 ± 0.0583	0.0562 ± 0.0113
Serum	0.2500 ± 0.0525	0.1165 ± 0.0565	0.0562 ± 0.0106
Clot of plasma	0.4957 ± 0.1358	0.2320 ± 0.0690	0.1657 ± 0.0184
Clot of Serum	0.5014 ± 0.1497	0.2430 ± 0.0721	0.1646 ± 0.0181

For R.A. patients samples in Jizan and Jeddah cities. It has been found that in Jizan city, the highest concentration of element zinc was in clot serum of patients (0.2430 ppm), followed by clot of plasma zinc concentration is (0.2320 ppm) and then in blood zinc concentration is (0.1895 ppm) and plasma zinc concentration is (0.1245 ppm) followed by serum zinc concentration (0.1165 ppm).

In case of patients R.A Jeddah city, the study showed that highest zinc concentration in clot of plasma is (0.1657 ppm) while lowest concentration of Zn element in plasma and serum is (0.0562 ppm).

The study has proved low concentration of zinc in patients with rheumatoid arthritis compared healthy and more low zinc concentration in patients R.A. Jeddah compared to patients R.A. Jazan. zinc concentration in blood is low in R.A. Jeddah city patients was (0.1610 ppm) compared to concentration of zinc in blood of R.A. Jizan City patients was (0.1895 ppm) and zinc concentration in blood of healthy was (0.4042 ppm).

The study showed that zinc concentration in plasma of R.A. Jeddah city patients is (0.0562 ppm), It is lower compared to zinc concentration in plasma of R.A. Jizan city patients is (0.1245 ppm) and zinc concentration in plasma healthy is (0.2314 ppm).The study showed that zinc concentration in serum of patients R.A. Jeddah city (0.0562 ppm) is lower compared to serum of patients R.A. Jizan city (0.1165 ppm) and zinc concentration in serum of healthy (0.2500 ppm).

The study has shown that:

i. concentration of zinc in clot of serum R.A. Jeddah city patients is (0.1646 ppm), it is lower compared to concentration of zinc in Clot of serum R.A. Jizan City patients is (0.2430 ppm) and zinc concentration in Clot of serum of healthy is (0.5014 ppm).

ii. zinc concentration in clot ofplasma of patients R.A. Jazan city is (0.1657 ppm), it is lower compared to concentration of zinc in clot of plasma patients R.A. Jeddah city is (0.2320 ppm) and zinc concentration in Clot ofplasma for healthy is (0.4957 ppm).

This can be explained that trace element zinc has a significant role in antioxidant protection and immunity. It constituents in antioxidative enzymes, zinc in cytoplasmic superoxide dismutase (SOD) [23]. which is an important antioxidant in serum. Also, Zinc is important in the maintenance of proper immune response [24].

In inflammatory conditions, such as rheumatoid arthritis (RA), there are alterations in blood and plasma concentrations of these micronutrients. During acute inflammation serum zinc decreases [25].

Factors that have an effect on stripping voltammetry of Zinc element in standard solutions of, healthy and rheumatoid arthritis patient samples in Jeddah and Jizan cities:

As has been studied the effect of each of (deposition time, deposition potential, pulse time, pulse amplitude, voltage step, voltage step time, drop size, Equilibration time) on each of voltage and current of behaviour of electrical for zinc element under study to identify the best conditions and factors that effect on determinationof concentration Zn element using this technique.

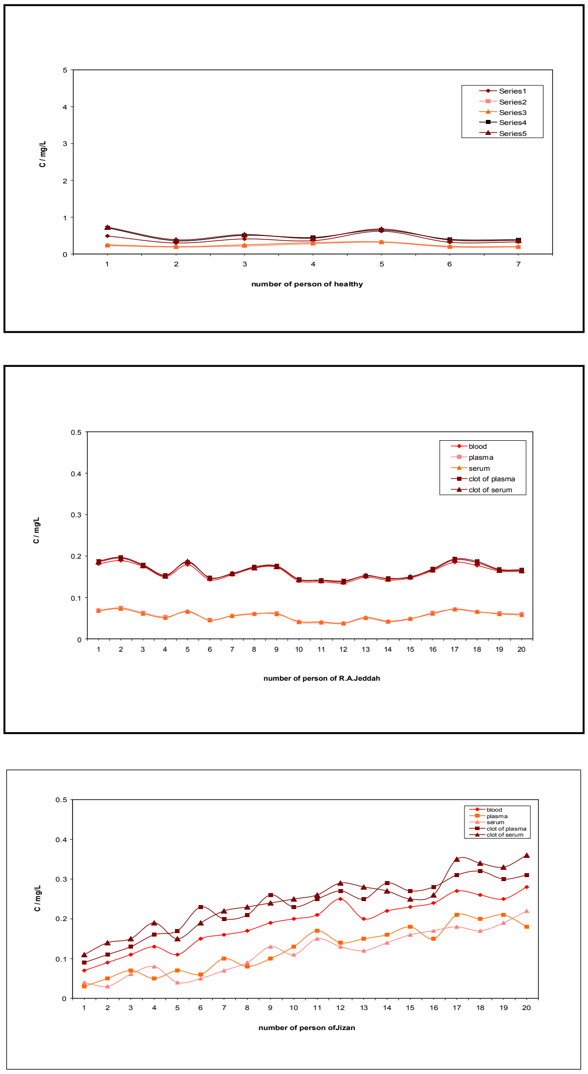

Fig. (1): Histogram of Znconcentration for:
a) Healthy. b) R.A. patient of Jeddah city. c) R.A. patient of Jizan city in (Blood, Plasma, Serum, Clot of Serum and Clot of Plasma).

Deposition time:

The study demonstrated that increase in deposition time (0 - 300 sec.) of zinc element in case of standard solution and samples from healthy and R.A. patients of Jeddah and Jizan cities leads to increase value of current stripping anodic in each of standard solution (0 - 29uA), extracted samples from healthy (3-54 nA) and rheumatoid arthritis patients in Jeddah (3 – 26 nA) and rheumatoid arthritis patients in Jizan (2 – 24 nA) in Figure (2).

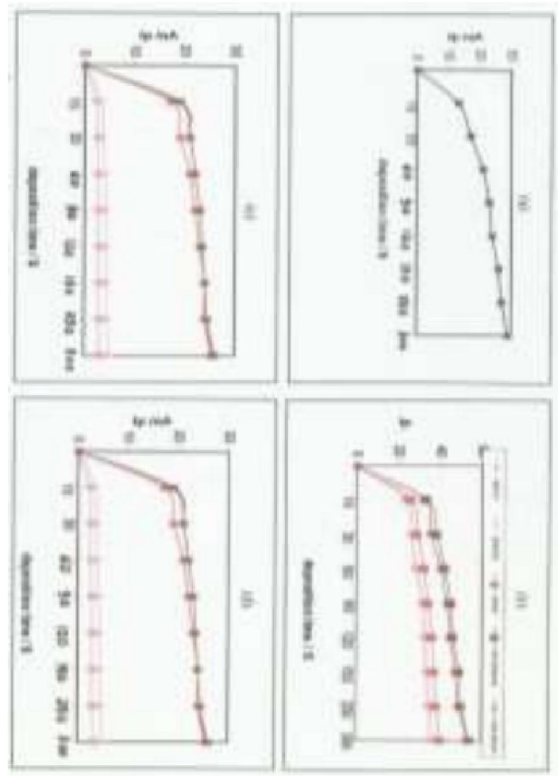

Fig. (2): The Zn Ip high depend on the deposition time for A) std. solution. B) Healthy. C)R.A. Jeddah city patients. D) R.A. Jizan city patients.

While value of pulse voltage was (-0.95V) in Figure (3), it is not affected by increase deposition time and remains constant, and in case of standard solution and samples from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities, where the study showed that best time of deposition time can be used to determine the concentration of zinc element in this study is (90 sec).

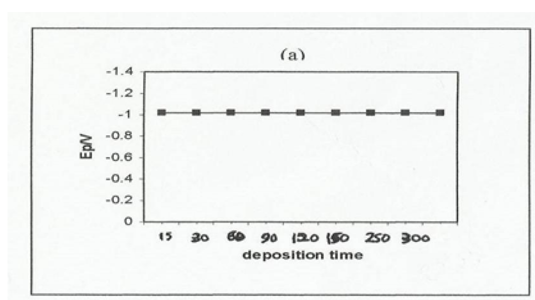

Fig. (3): Relationship between the Zn Ep.(of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and deposition time.

Deposition potential:

The study showed an increase in deposition potential (-1.35 – - 0.95 V) for zinc element in case of standard solutions and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities that lead to increase value of the current anodic stripping in each of standard solution (0 - 29uA) and healthy extract samples (nA 0-54) and R.A. patients of Jeddah city (3 - 26nA) and R.A. patients of Jizan city (2 - 24nA) figure (4).

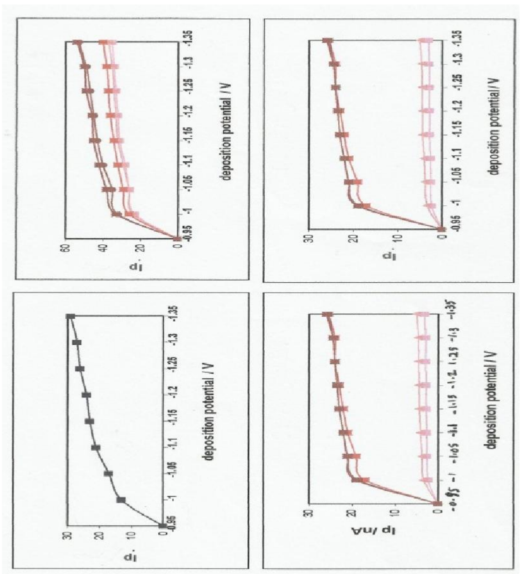

Fig. (4): The Zn Ip high depend on the deposition potential for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

While value of pulse voltage is (-0.95V) in Figure (5),it is not affected and remains constant by increase deposition potential and in case of standard solution and extract samples from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities where this study showed that best deposition potential can be used to determine concentration of zinc element in this study is (-1.15V).

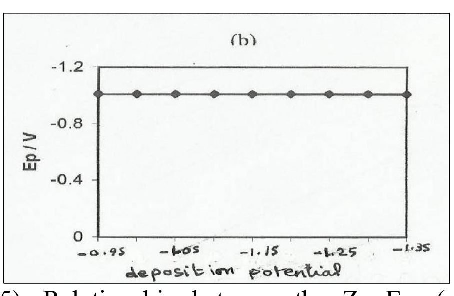

Fig. (5): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and deposition potential.

Pulse time

The study show increase in pulse time (0.01 - 0.09 sec.) of zinc element in case of standard solutions and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities lead to decrease in anodic stripping current value in each of standard solution (0 - 29uA) and samples extract from healthy (0 – 54nA) and rheumatoid arthritis patients in Jeddah city (3 - 26nA) and rheumatoid arthritis patients Jizan city (2 - 24nA) figure (6).

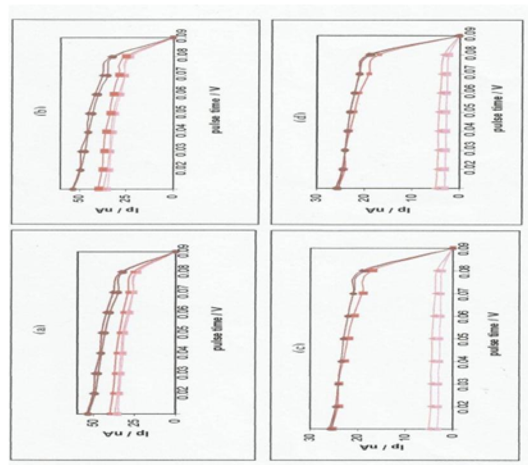

Fig. (6): The Zn Ip high depend on pulse time for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

While value of pulse potentials (-0.95V) Figure (7), it is remains constant by the increase of pulse time and in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and rheumatoid arthritis patients Jizan cities, where the study show that best pulse time can be used to determine concentration of zinc element in this study is (0.04 sec).

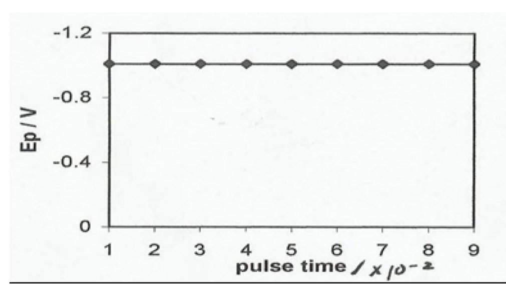

Fig. (7): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and pulse time.

Pulse amplitude

The study shows increase in pulse amplitude (0.01 - 0.09V) for zinc element in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities lead to a decrease in anodic stripping current value in each of the standard solution (0 - 29uA) and samples extract from healthy (0 – 54 nA) and rheumatoid arthritis patients in Jeddah city (3 -26nA) and rheumatoid arthritis patients Jizan city (2 - 24nA) Figure (8).

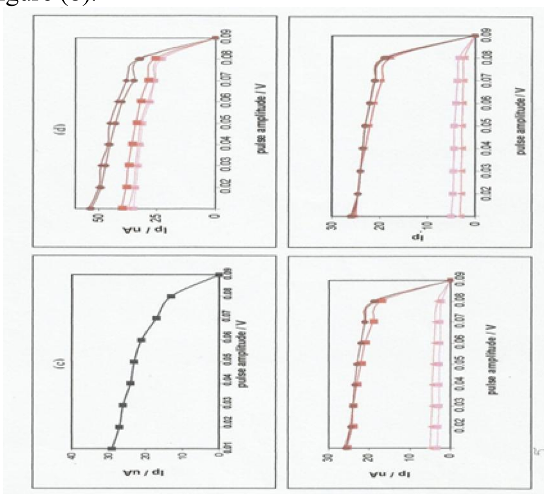

Fig. (8): The Zn Ip high depend on pulse amplitude for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients

While value of pulse potential is (-0.95V), it is not affected and remains constant by increase of pulse amplitude and in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities Figure (9), where the study shows that best pulse amplitude can be used to determine concentration of zinc element in this study is (0.05 V).

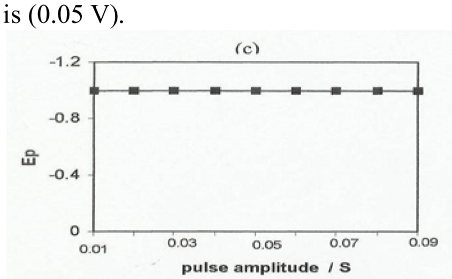

Fig. (9): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and pulse amplitude.

Voltage step:

The increase in voltage step (0.001 - 0.009V) for zinc element in case of standard solution and samples extract from healthy and patients with rheumatoid arthritis in Jeddah and Jizan cities leads to increase anodic stripping current value in each of standard solution (0 - 29 uA) and samples extract from healthy (0 – 54 nA) and rheumatoid arthritis patients in Jeddah city (3 - 26 nA) and rheumatoid arthritis patients in Jizan city (2 - 24 nA) figure (10). While the value of pulse voltage is not affected by increase voltage step and remain constant (-0.95V) and in case of standard solution and samples extract from healthy and patients with rheumatoid arthritis in Jeddah and Jizan cities figure (11), also study show that best voltage step can be used to determine concentration of zinc element in this study is (0.005 V).

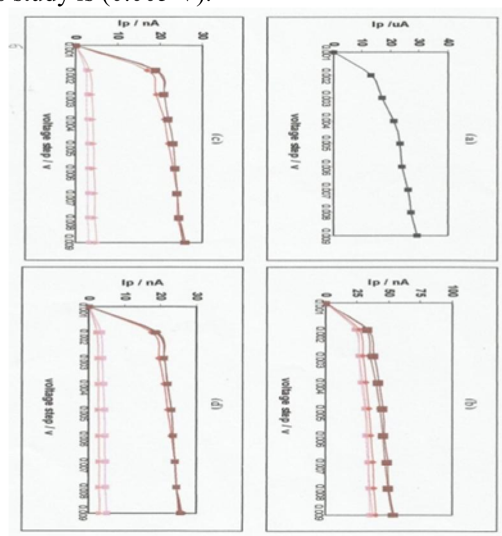

Fig. (10): The Zn Ip high depend on voltage step for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

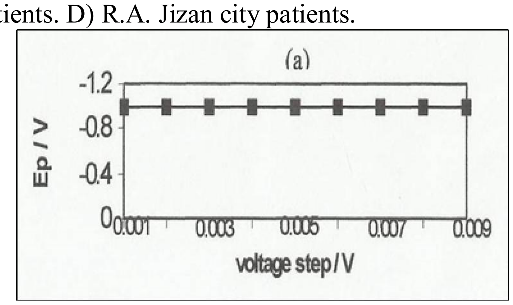

Fig. (11): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and voltage step.

Voltage step time:

The study show increase in voltage step time (0.06 - 0.14 sec) for zinc element in case of standard solution and extract samples from healthy and rheumatoid arthritis patients in Jizan and Jeddah cities and lead to decrease Anodic stripping current value in each of standard solution (0 - 29uA) and samples extract from healthy (0 - 54 nA) and rheumatoid arthritis patients in Jeddah city (3 - 26nA) and rheumatoid arthritis patients in Jizan city (2 - 24nA) in figure (12).

While value of pulse voltage is (-0.95V), it is not affected by increase voltage step time and remains constant in all studied samples Figure (13). Where the study showed that best voltage step time can used to determine concentration Zinc element in this study is (0.1 sec).

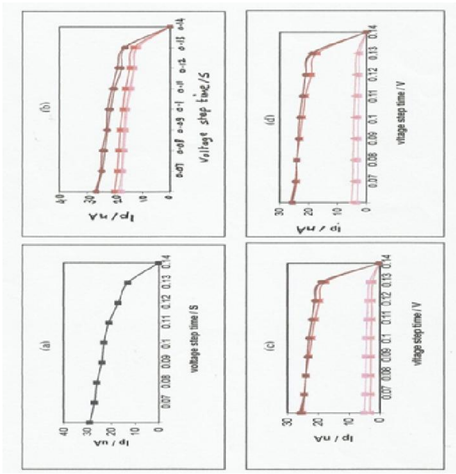

Fig. (12): The Zn Ip high depend on voltage step time for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

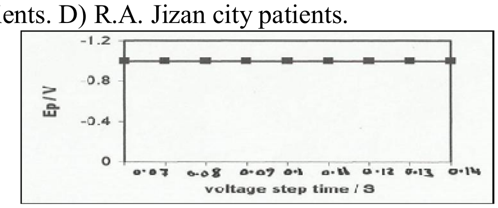

Fig. (13): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and voltage step time.

Size of hanging mercury electrode drop

The study show increase in size of hanging mercury electrode drop (1-9) for zinc element in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities lead to increase value of current Anodic stripping in each of standard solution (0 – 29uA) and samples extract from healthy (0 – 54nA) and rheumatoid arthritis patients in Jeddah city (3 - 26nA) and Jizan city (2 – 24nA) in figure (14).

While value of pulse voltage is (-0.95V), it is not affected by increase size of mercury drop and remain constant and in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities in figure (15). Where the study show that best size of mercury drop can be used to determination zinc element concentration is (4).

Equilibrium Time:

The study show that increase in equilibrium time (2 - 18 sec.) of zinc element in case of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities lead to decrease in current value Anodic stripping in each of standard solution (0 - 29uA) and samples extract from healthy (0 – 54nA) and rheumatoid arthritis patients in Jeddah city (3 - 26nA) and rheumatoid arthritis patients in Jizan city (2 - 24nA) in figure (16).

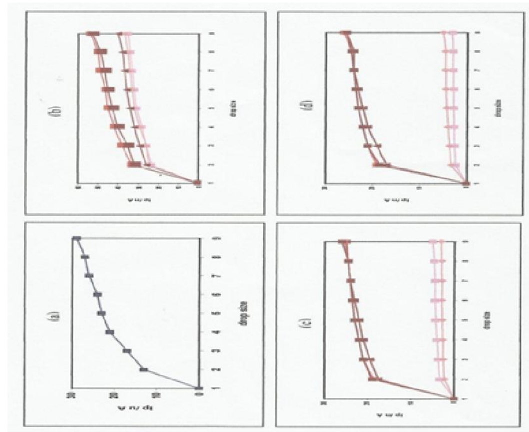

Fig. (14): The Zn Ip high depend on drop size for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

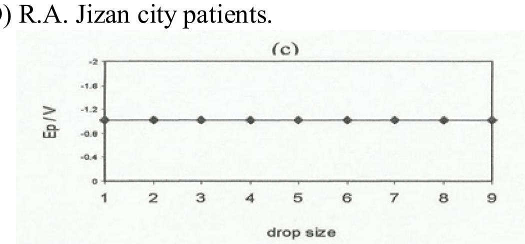

Fig. (15): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, and R.A. Jizan city patients) and drop size.

While value of pulse voltage is (-0.95V), it is not affected by increase of equilibrium time and remains constant, and in case of those solutions for each of standard solution and samples extract from healthy and rheumatoid arthritis patients in Jeddah and Jizan cities in figure (17). Where the study show that best equilibrium time can be used to determine concentration of zinc element is (10sec).

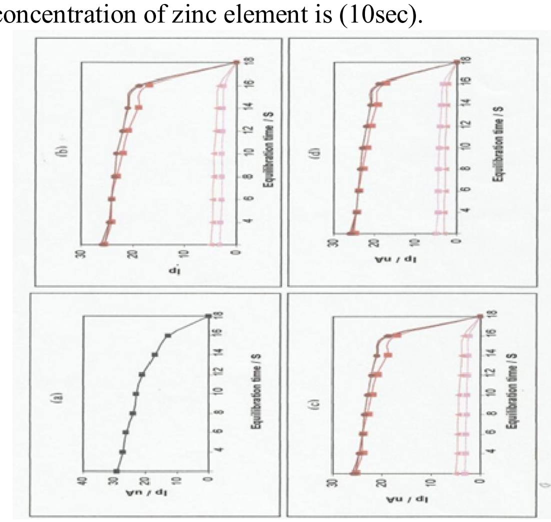

Fig. (16): The Zn Ip high depend on equilibrium time for A) std. solution. B) Healthy. C) R.A. Jeddah city patients. D) R.A. Jizan city patients.

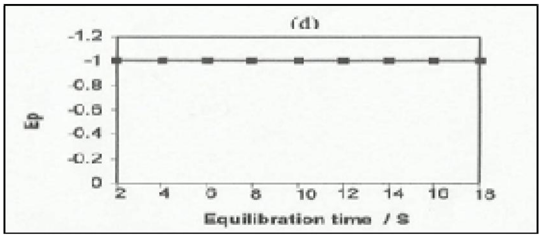

Fig. (17): Relationship between the Zn Ep. (of std. solution, Healthy, R.A. Jeddah city patients, R.A. Jizan city patients) and equilibrium time.

In Figure (18, 19) describes voltammogram stripping for zinc element of extracted samples from rheumatoid arthritis patients (Plasma, Serum, Blood, Clot of serum, Clot of plasma) in Jeddah and Jizan cities.

Anodic stripping voltammetry (ASV) is a powerful technique used for rapid determination of trace levels of metal ions. For determination of zinc by ASV [26, 27].

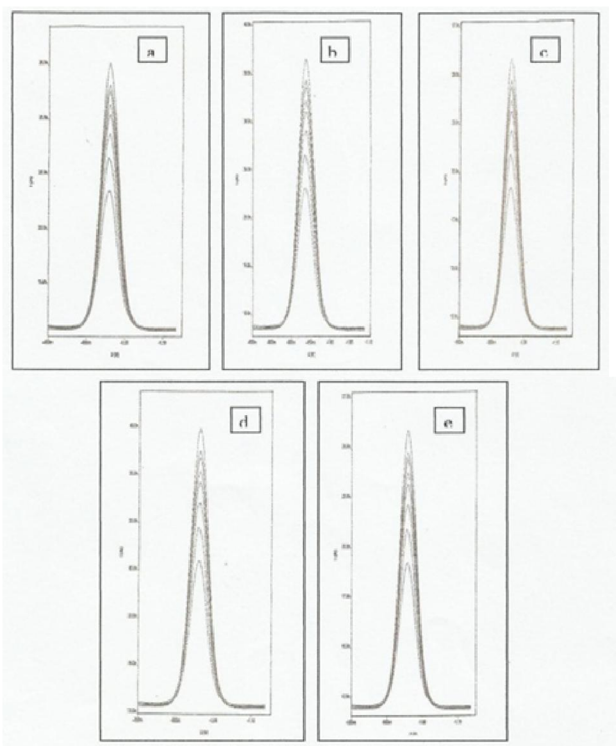

Figure (18): Voltammogram stripping for zinc element of extracted samples from rheumatoid arthritis patients (Plasma, Serum, Blood, Clot of serum, Clot of plasma) in Jizan city.

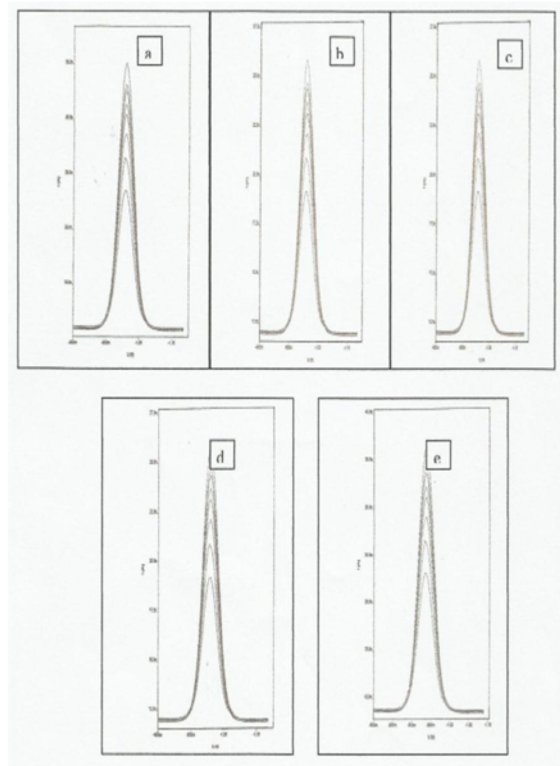

Figure (19): voltammogram stripping for zinc element of extracted samples from rheumatoid arthritis patients (Plasma, Serum, Blood, Clot of serum, Clot of plasma) in Jeddah city.

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