Support vector machine for simultaneous determination of ultra trace concentrations of copper and cadmium in serum of patients with chronic hepatitis by adsorptive stripping voltammetry

AbdulSamad A. Marghilani
Dean of Scientific Research, Northern Border University, KSA
E-mail: srd.nbu@gmail.com

Ahmed. F. Al-Hossainy
[2]Department of Chemistry, Faculty of Science, Northern Border University, KSA
E-mail: ahmed73chem@yahoo.com

R. F. Mansour
[2]Department of Computer Science, Faculty of Science, Northern Border University, KSA
E-mail: romanyf@aun.edu.eg

Abstract

The purpose of this study is to establish a simple model and use the clinical data to predict the interferon efficacy. This model is a combination of Feature Subset Selection and the Classifier using a Support Vector Machine (SVM). The study indicates that when five features have been selected, the identification by the SVM is as follows: the identification rate for the effective group is 85%, and the ineffective group 83%.Serum trace elements concentrations and their ratios are frequently reported to be a good marker for diagnosing various diseases; one of these diseases is chronic liver diseases. This study undertaken to simultaneous determine of Cu^{2+}, and Cd^{2+} concentrations in sera from viral hepatitis (B and C) patients by adsorptive stripping voltammetry using 3-aminophthalhydrazide (luminol) as a complex agent and classification with support vector machine SVM. Donor's hepatitis patients and healthy persons were selected from different environmental areas, including Arar, Al-auiqilh and Rafha as unpolluted areas, and Al-Jouf , Tarif and Al- Qurayyat as a polluted areas. Hepatitis patients from polluted areas exhibited higher Cu^{2+} and Cd^{2+}concentrations in their serum than those from the other areas. Patients with hepatitis B show higher levels of Cu^{2+}, and Cd^{2+} in their serum than those with hepatitis C. Copper and cadmium presented at higher level in patient serum than in healthy ones. In this study the optimum reaction parameters and conditions studies are investigated. The calibration graphs were linear in the concentration range of 0.3– 142.5 and 0.065–60.0 ng/mL for copper and cadmium, respectively. The limit of detection of the method was 0.038ng/mL for Cu^{2+} and 0.013 ng/mL for Cd^{2+}. The interference of some common ions was studied and it was concluded that application of this method for the determination of Cu^{2+} and Cd^{2+}in the healthy control and hepatitis patient's serum led to satisfactory results.

Keywords: Support Vector Machine; adsorptive stripping voltammetry; chronic hepatitis; heavy metal.

1. Introduction

In this study, a simple model was established to predict the efficacy of interferon therapy for Chronic Hepatitis C (CHC) patients. The first step for the model is the selection of clinical markers (feature subset) that are the key to the classification task and then assessed by Fisher Criterion to determine the feature significance. The second step is to classify and identify the two (effective/ineffective) groups by means of the Support Vector Machine (SVM) using selected clinical markers. SVM is a linear machine that is trained to find the optimal discrimination hyperplane of a given data set, Basically it works with two groups of problems [1]. In the last few decades the analytical study of concentrations the main and trace elements in biological and human samples have become very important [2]. Occupational diseases, poisoning, and environmental diseases are accurately diagnosed by using trace elemental analysis of human biological samples and the state of health can be characterized with the analytical results [3]. The importance of these examinations is attested to by the fact that there are several trace elements in the human body that are important in the biochemical processes. An excess or absence of these essential trace elements causes serious problems in the physiology of the body. Thus, it is very important to check regularly trace element concentrations in the body. Trace element analysis of biological samples is a considerable challenge to analytical chemists. Because of the large amount of organic substances in the samples, digestion or ashing is required prior to analysis and the low concentrations of the measured elements demand

well-organized, special sample pretreatment and sensitive analytical methods and equipment. Thus, trace elements comprise metals in biological fluids at concentrations <1 µg/g of wet weight [4]. Among the trace elements, iron, copper, and zinc are involved in the function of several enzymes and are essential for maintaining health throughout life; lead and cadmium are non-essential toxic metals [5-6].
Copper is essential for a variety of biochemical processes [7] and is needed for certain critical enzymes to function in the body [8]. Copper is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as zinc and molybdenum, and other body functions. Copper also causes the ischaemic heart disease, anaemia, bone disorders [9]. The maximum tolerable daily intake for copper is 0.5 mg/kg body weight [10].

Serum concentration of copper plays an important role in the interrelation among immunoglobulin's IgA, IgM and IgG (Immunolobulins are antibodies in immune system and some of types IgA, IgM and IgG) in subjects with various forms of liver diseases [11]. Copper accumulation in fibro tic livers caused by chronic HCV infection may contribute to hepatic injury. The hepatic copper contents increased with the progression of hepatic fibrosis and concluded that the presence of copper may enhance HCV infection [12]. Serum trace elements including copper in sera of patients with viral hepatitis (A, B, C, D, E) cases and the controls. They have shown elevation in copper levels and have suggested that this probably resulted from defense strategies of organism and induced by hormone-like substances [13].
Cadmium is considered as one of the most toxic elements for living organisms. The environmental persistence of the metal in concert with their intensive use by modern society has, over the years, created a concentration in the biosphere, and continuous exposure to low levels of cadmium may result in bioaccumulation and health effects, by both occupational and environmental exposition [14]. Human biomonitoring is a useful tool in environmental medicine to assess and evaluate the background levels of internal dose of environmental pollutants in general population, population groups and individuals through measurements of those chemicals, or their metabolites in human specimens, such as blood and urine [15]. The determination of copper and cadmium in biological materials poses several problems mainly due to the low concentrations of these metals, the great variation of the matrix from sample to sample and contamination[16].

Chronic exposure to cadmium can result in chronic obstructive lung disease, renal disease, and fragile bones. Protect children by carefully storing products containing cadmium, especially nickel-cadmium batteries. Symptoms of chronic exposure could include alopecia, anemia, arthritis, learning disorders, migraines, growth impairment, emphysema, osteoporosis, loss of taste and smell, poor appetite, and cardiovascular disease. [17].

Adsorptive stripping voltammetry (AdSV) is an extremely sensitive electrochemical technique for measuring trace metal concentrations in whole blood [18-21]. The method is based on the formation and interfacial accumulation of a metal complex on the working mercury electrode and subsequent measurement of the reduction peak of the accumulated complex. Numerous authors have used this technique in order to measure trace element concentrations in different matrices, human hair [22], and human blood [23].

3-aminophthalhydrazide (Luminol) behavior in aqueous NaOH and H_2SO_4 solutions has been studied by steady-state fluorescence and time-resolved single-photon counting techniques [24]. Also, a selective method is presented for the simultaneous determination of copper and cadmium in food samples by adsorptive stripping voltammetry. In preliminary studies, it has been proven that the copper and cadmium react with luminol, giving rise to the formation of these complexes. These complexes have adsorptive characteristics on hanging mercury drop electrode (HMDE) and can be reduced in a reduction step.

The objective of this work is to propose a sensitive adsorptive stripping voltammetric method that developed for the simultaneous determination of low levels of copper and cadmium in serum of patients with chronic hepatitis C and B, and compared the results with those of healthy group. In addition, the effect of environmental areas on serum metal levels was studied. The procedure is based on the reduction of the complexes of those metal ions with luminol after accumulation at the surface of a hanging mercury drop electrode.

2. Materials and methods

Study areas

Locations with industrial activities and other sources that emit various metals to the environment are known to contribute a great deal of metals to the inhabitants through a variety of routes. In our study, chosen the following six sites from two different environmental locations:

1- Unpolluted areas include Arar, Al-auiqilh and Rafhaa, Cities located north of the kingdom, which lies about 1,100 km from the city of Riyadh near the Iraqi border.
2- Polluted areas include Al-Jouf , Tarif and Al- Qurayyat, where some pollution activities were presented. Population samples were divided into two different groups to assess the degree of exposure of selected metals. The groups were based on gender, donor locations, and types of hepatitis. These were normal (healthy) and exposed (patients) male and female groups of population. Consent was obtained for all patients and healthy donors.

Patients and healthy donors

In all sixty patients with viral hepatitis (30 females sand 30 males) aged between 25 and 74 years, as well as sixty healthy individuals (30 females, 30 males) aged between 25 and 75 years were chosen for our study. Patients were classified as 40 hepatitis C(HCV) and 20 hepatitis B(HBV). All sufferings of patients from the disease were mild. Diagnosis of hepatitis is based on symptoms, physical findings, as well as blood tests for liver enzymes and viral antibodies. Hepatitis C (HCV) and B (HBV) were determined in clinical laboratories of hospitals by third generation micro-particle (ELISA). The following markers were used: 1-hepatitis B surface antigen (HBsAg) by ELISA assay using Merux HBsAg version 3 kit and 2-hepatitis C antibodies (Anti-HCV Ab) by ELISA assay using Merux Anti-HCV version 4 kit.

Blood sample collection and preparation

Blood samples (5ml each) of patients were collected by venous puncture from hospital pathogenesis, in Arar educational, Prince Abdul Aziz bin mosaed, Central Rafhaa, Prince Abdul Rahman Al-Sudairi Jouf and Al-Qurayyat hospital. Blood samples of healthy donors were collected from the same areas of patients. All donors were not administered by antiviral treatment before this study. The donor puncture site was cleaned to remove any expected contamination before sampling. Separate and disposable sterilized plastic syringes were used for blood collection. The blood sample was left standing for 1h to coagulate; serum was separated at 2500rpm centrifugation for 10 min, transferred to 5mLpolystyrene tube, and stored at -18^{o}C until analysis.

Reagents

All chemical reagents were of analytical grade and were purchased from Merck (Germany). All solutions were prepared with double distilled water. Stock standard solutions of Cu^{2+} and Cd^{2+} (1000µg/mL) were prepared by dissolving 0.3802 and 0.2740 g of $Cu(NO_3)_2$ and $Cd(NO_3)_2$ salts in water and diluting to 100 ml in a volumetric flask every week. A $1.0x10^{-3}$ M solution of luminol (Sigma) was prepared by dissolving the appropriate amount of luminol in NaOH (Merck) 1.0 M. Borate buffer solutions were used for fixing the pH in the range of 5.5–10.0.

Instrumentation differential pulse adsorptive stripping voltammetry (DPA$_d$SV) technique

For the voltammetric measurements, a fully computerized Electrochemical Trace Analyzer Model 273 APAR (EG&G) was used interfaced with an electrode assembly model 303A (EG&G) of a static mercury drop electrode (SMDE) in the hanging mercury drop electrode (HMDE) mode (A=$2.6x10^{-2}$ cm^2). Stirring was performed with a Teflon coated bar at about 400 rpm using a magnetic stirrer (KIKA Labortechinik, Germany), Ag/AgCl (in saturated KCl) reference electrode, and auxiliary electrode of a platinum wire. At the beginning of the experiment, the solutions were deoxygenated with high purity nitrogen for 10min, whereas before each analysis step the solution was deoxygenated for 2 min.

Sample preparation for analysis

One milliliter serum sample was wet digested in covered Teflon beaker containing a 10mL (1:3) $HNO_3/HClO_4$ acid mixture [25]. The digest was transferred in to a 15ml pre-cleaned measuring flask, diluted to the mark with double distilled water, and stored in glass bottles for analysis. Blank solution was treated and prepared in the same way as the samples. Each sample and each blank were prepared in triplicate.

Recommended procedure

A sample solution (10 mL), containing 0.10 M Borate buffer (pH 7.5), $1.0x10^{-4}$M luminol, and different concentrations of Cu^{2+} and Cd^{2+} were transferred to the voltammetric cell and purged with nitrogen for 10 min. The accumulation potential (-0.01 V) was applied to a fresh mercury drop for 60s while the solution was stirred. Following the accumulation period, the stirring was stopped, and after 10s the voltammograms were recorded by applying a negative going potential. Each scan was repeated four times with a new drop for each analyzed solution and the mean of the voltammograms was obtained. The currents of Cu^{2+} and Cd^{2+} were used as a measure of ion concentrations. All data were obtained at ambient temperature. The experimental conditions for the simultaneous determination of Cu^{2+}, and Cd^{2+} by DPA_dSV are presented in Table 1.

Table 1 Experimental condition for the simultaneous determination of copper and cadmium in blood serum and reference material (WMP8435) by differential pulse adsorptive stripping voltammetry (DPA_dSV) and differential pulse anodic stripping voltammetry (DPASV).

Metals	Method	Conditions of Methods				Reference material: Whole Milk Powder WMP8435	
		Dp (mV)	Fp (mV)	Dt (s)	Sc (mV/s)	Certified (ppm)	Measured
Cu^{2+}	DPASV	-150	-0.	90	10	0.46	0.4587
	DPA_dSV	-520	-0.90	60	100		0.460
Cd^{2+}	DPASV	-600	+0.05	150	10	0.0002	0.00019
	DPA_dSV	-690	-0.90	60	100		0.0002

Supporting electrolyte: HMDE (Hanging mercury drop electrode); Dp, deposition potential; Fp, final potential; Dt, deposition time; Sc, potential scan rate.

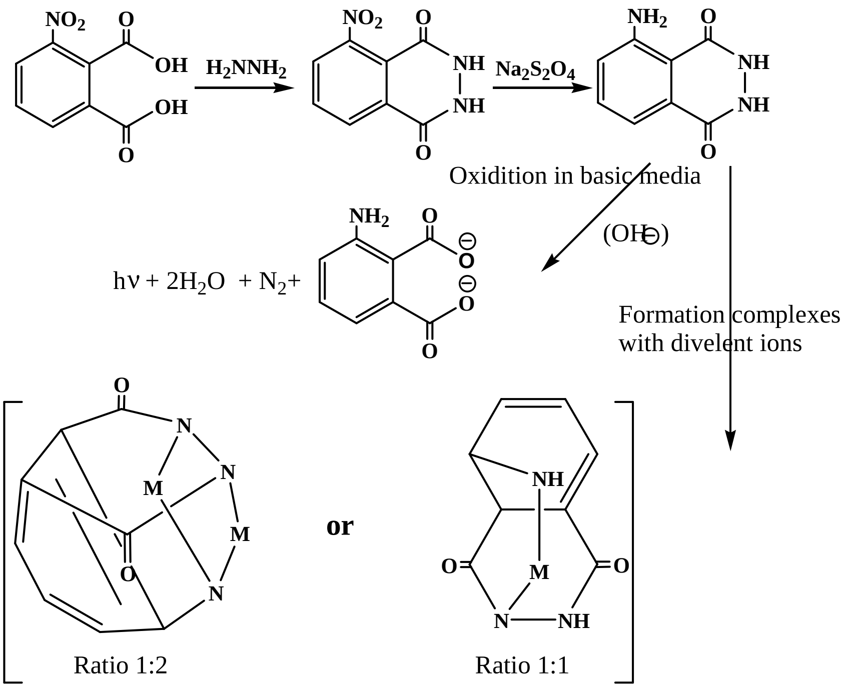
Quality assurance

The reliability of the procedure for determining ultra trace Cu^{2+} and Cd^{2+} in blood serum by DPA_dSV and DPASV has been checked by analyzing standard reference materials. The standard reference material Whole Milk Powder 8435(WMP8435) from the National Institute of Standard and Technology in Canada was analyzed for copper, and cadmium. The results agree with in with certified values (Table 1). The validity of the method was further determined by cross-method check, spike recovery, and replicate analysis.

3. Results and discussion

Presumptive test for blood

Sodium perborate (3.5 g of Aldrich product number 24,412-0) is added to 500 mL distilled water and thoroughly dissolved. Sodium carbonate (25 g) and luminol (9.5 g) are then added and dissolved. The solution is allowed to stand for five minutes to allow any undissolved chemicals to settle. The solution is then decanted into a plastic spray bottle and is ready to use. It should be applied as a fine mist on the surface to be tested. True bloodstains will luminesce with an even glow that will last for several seconds; for better viewing, the scene should be as dark as possible [26]. It is significant to note that this test is only presumptive, since it is the iron in the heme which catalyzes the oxidation and subsequent light emission. The presence of copper as a contaminant will accelerate oxidation. Try the spray on a freshly cleaned copper penny.



Where M= Cu^{2+} or Cd^{2+}

Scheme 1 Synthesis, analysis and oxidation of Luminol ligand in basic solution

Luminol can act as a bidentate chelating agent, coordinating with both a metal ion at the amino group and one of the hydrazide carbonyls to form a 6-membered ring. The divalent metals of Cu^{2+} and Cd^{2+} can form stable 1:2 complexes with schiff bases such as luminol (Scheme (1)) [27].

Luminol has some drawbacks that may limit its use in a crime scene investigation, such as, chemiluminescence can also be triggered by a number of substances such as copper or copper-containing alloys, and certain bleaches; and, as a result, if a crime scene is thoroughly cleaned with a bleach solution or horseradish, residual cleaner will cause the entire crime scene to produce the typical blue glow, effectively camouflaging any organic evidence, such as blood. It will also detect the small amounts of blood present in urine, and it can be distorted if animal blood is present in the room that is being tested. And also reacts with fecal matter, causing the same glow as if it were blood.

According to our knowledge, simultaneous determination of trace elements in blood serum has been described with other methods. Table 2 shows some advantages of present work compared with previous studies.

Adsorptive and Voltammetric Characteristics of the Cu^{+2} and Cd^{+2}-Luminol Complexes

Preliminary experiments were carried out to identify the general features which characterize the behavior of the metal ions–luminol systems on a hanging mercury drop electrode. Fig.(1a) displays adsorptive stripping voltammograms of mixture of metal ions (Cu^{2+} and Cd^{2+}) and luminol system between -0.3 and -0.9 V in a solution containing different concentrations of these metals under optimum conditions. Two separated peaks for the reduction of cadmium and copper complexes with luminol were observed that was due to the difference in the thermodynamic stability of their complexes. Comparison of the voltammograms revealed that the height of the copper and cadmium peaks depends not only on the duration of the pre-concentration step, also the stripping initial potential, which revealed the adsorptive nature of the response [28].

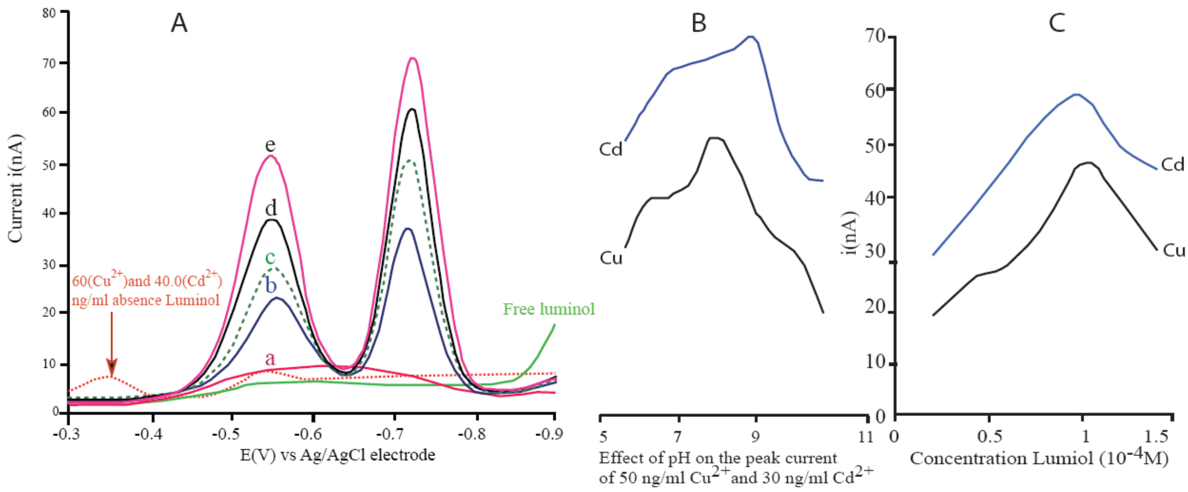


Fig. (1A-C). Adsorptive stripping voltammetry for Cu^{2+} and Cd^{2+}-luminol system; (a) 0, 0 ng/mL, (b) 10, 10 ng/mL, (c) 20, 15 ng/mL, (d) 25, 20 ng/mL, (e) 35, 25 ng/mL, respectively, at optimal conditions: 0.10 M borate buffer solution, pH 7.5, luminol concentration of $1x10^{-4}$ M, E_{acc} of -100 mV, t_{acc} = 60 s and scan rate of 100 mV/s.

The influence of pH (Fig.1B) by using borate media on the cathodic stripping peak currents of copper and cadmium was studied in the pH range of 5.5–10.0. It was found that at pH 7.5 the peak currents of cadmium and copper were at their maximum values. Thus, pH 7.5 was adopted for further studies. An optimum luminol concentration of $1x10^{-4}$ M was selected for further experiments (Fig. 1C).

Table 2 Results of the comparison of the merit of the present work with the other studies

Method	Real samples		LOD (µg/L)		LR (µg/L)		Interferences	References
			Cu	Cd	Cu	Cd		
Square wave cathodic stripping voltammetry	Water river Nile (Egypt)	Working electrode HMDE	0.017	-	0.4-50	-	Fe^{3+}, Cd^{2+}	Ahmed F.Al-Hossainy (2010) [29]
Square wave cathodic stripping voltammetry	Water		0.012	0.028	0.05-10	0.12-40	Ascorbic acid	Beltagi and Ghoneim (2009) [30]
Differential pulse cathodic adsorptive stripping voltammetry	Water river Nile (Egypt)		0.04	-	0.6-30	-	Fe^{3+}, Cd^{2+}	Ahmed F.Al-Hossainy (2010) [29]
Differential pulse cathodic adsorptive stripping voltammetry	Blood serum		0.038	0.014	0.5-110	0.8-70	CN	Present work
Differential pulse cathodic adsorptive stripping voltammetry	Food		0.04	0.02	0.5-105	0.8-70	CN	Shahryar et al. (2011) [24]
Differential pulse anodic stripping voltammetry	Sugar beet		0.2	0.5	0.1µg/l to few mg/ml		-	Sancho et al. (2005) [31]
Differential pulse anodic stripping voltammetry	Blood serum		0.2	0.5	0.1µg/l to few mg/ml		-	Rashed et al. (2010) [1]
Adsorptive stripping voltammetry	tap water and synthetic samples		0.4	0.9	0.5-100	1-100	Zn^{2+}, Ba^{2+}, Mn^{2+}, Ca^{2+}	Babaei et al. (2007) [32]
Adsorptive voltammetry	Natural water		0.87	0.96	-	-	Zn^{2+}, Organic Matters (TritonX-100)	Iliadou et al. (1997) [33]
Anodic stripping voltammetry	Glazed ceramic		2.7	0.25	0-200	0-200	-	Jakmunee et al. (2008) [34]
Adsorptive cathodic stripping voltammetry	Food		-	1.180	1-50	10-200	Ni^{2+}, Cr^{3+}, Co^{2+}	Ensafi et al. (2006) [35]

According to Table 2, it was shown that the calculated detection limits of some of these methods are reported in comparison with proposed method not to be good. The given detection limits of the elements under investigation revealed that the proposed scheme of analysis under the optimal conditions is very sensitive and useful for ultra trace determination of copper and cadmium elements. The detection limits LOD (µg/L) of the investigated metals, defined as the metal concentration yielding an analytical peak equal to the minimum detectable one, can be calculated as; LOD=5(S_d/m), where, S_d is the standard deviation of the blank and m is the slope of the calibration line [36].

The pre-concentration time is short (60 s), the limit of detection and linear dynamic range of method is very good. The most of cations and anions do not interfere by determination of copper and cadmium. It was found that there was not any inter-metallic effect between copper and cadmium ions in this system.

The advantageous sensitivity and selectivity makes this proposed method a favorable competitor to the previously published methods for the determination of copper and cadmium. In this work, a new sensitive and rapid adsorptive voltammetric method for the determination of ultra trace levels of copper and cadmium in serum of patients with chronic hepatitis C and B was developed based on adsorptive stripping voltammetry of copper and cadmium complexes with luminol.

Environmental results

Human health is directly related to the environment. The number and amount of environmentally damaging toxic substances entering the environment has increased rapidly due to the expansion in industrial and transportation related activities in the last few decades. Some of these substances are trace elements and their compounds. They are widely distributed in air, water, and soils and therefore, human exposure is important either directly or via vegetables and animals.

Relationships of ultra Cu^{2+} and Cd^{2+} concentrations in serum of hepatitis patients and healthy control

The results of Cu^{2+} and Cd^{2+} concentrations in blood serum of healthy and hepatitis people as determined by adsorptive stripping voltammetry are presented in Table 3.

Table 3. Cu^{2+} and Cd^{2+}concentrations (**ppm**) in blood serum of healthy and hepatitis patients measured by differential pulse cathodic adsorptive stripping voltammetry.

Case and item Metals		Added (ppm)		Found (ppm)					
		Cu^{2+}	Cd^{+2}		Cu^{2+}±SD			Cd^{+2}±SD	
				Healthy n= 60	(HCV) n=40	(HBV) n=20	Healthy n= 60	(HCV) n=40	(HBV) n=20
				Unpolluted areas					
Arar City	Differential pulse cathodic adsorptive stripping voltammetry	0	0	0..946±0.381	1.1921±0.572	1..3204±0.423	0.0019±0.0007	0.0025±0.0012	0.0037±0.0034
		5	5	5..973±0.412	6..2079±0.308	6..2793±0.207	5.0021±0.0004	5.0023±0.0016	5.0036±0.0021
		10	10	11.021±0.207	11.203±0.279	11.267±0.333	10.0016±0.0006	10.0027±0.0027	10.004±0.0057
Al-auiqilh City		0	0	0.798±0.291	0..9563±0.424	1.089±0.402	0.0017±0.0002	0.0026±0.00023	0.0029±0.00102
		5	5	5.803±0.302	5..9831±0.503	6.103±0.364	5.0020±0.0006	5.0023±0.00030	5.0033±0.00143
		10	10	10.83±0.628	11.042±0.409	11.088±0.73	10.0019±0.001	10.0031±0.0056	10.0032±0.0024
Rafhaa City		0	0	0.828±0.178	1.0642±0.469	1.193±0.391	0.0017±0.0005	0.0023±0.00145	0.0033±0.0026
		5	5	5.810±0.208	6.1108±0.692	6.189±0.372	5.0015±0.0008	5.0028±0.00396	5.0029±0.0031
		10	10	10.806±0.372	11.079±0.849	11.064±0.79	10.0020±0.0011	10.0028±0.0044	10.003±0.00544
				Polluted areas					
Al-Jouf City		0	0	1.029±0.189	1.2631±0.3732	1..3838±0.362	0.0017±0.0004	0.0025±0.00051	0.00317±0.00072
		5	5	6.052±0.2791	6.2183±0.2964	6..3379±0.489	5.0018±0.0007	5.0029±0.00073	5.00353±0.00142
		10	10	11.163±0.381	11.316±0.5788	11.401±0.708	10.0020±0.0011	10.0023±0.001	10.0035±0.00262
Tarif City		0	0	1.257±0.367	1.4125±0.4463	1.727±0.4251	0.0023±0.0006	0.00319±0.0008	0.0041±0.00043
		5	5	6..302±0.281	6.3798±0.4762	6. 809±0.3752	5.0027±0.00084	5.0037±0.00104	5.00407±0.00063
		10	10	11.31±0.409	11.439±0.9319	11.779±0.593	10.0029±0.00104	10.0035±0.0016	10.0039±0.00101
Al-Qurayyat City		0	0	0.842±0.154	1.234±0.3428	1..5792±0.413	0.0014±0.0002	0.0023±0.00094	0.0057±0.00062
		5	5	5.860±0.205	6.609±0.2963	6.6860±0.5922	5.0017±0.0005	5.0026±0.00106	5.0061±0.00071
		10	10	10.831±0.721	11.25±0.7392	11.5894±0.739	10.0016±0.0008	10.002±0.00138	10.006±0.00102
Mean ± SD of Total				0..9504±0.173	1.1870±0.159	1..3821±0.238	0.00178±0.0003	0.00257±0.00033	0.00381±0.00102
Standard error of the mean (SE)				0.02234	0.02521	0.05321	0.000038	0.00005	0.00022
Minimum				0.7986	0..9563	1.0890	0.00140	0.00230	0.00290
Maximum				1.2570	1.4125	1.7270	0.00230	0.00319	0.00570

SD= Standar deviation, HCV= hepatitis C virus, HBC= hepatitis B virus

The mean Cu^{2+} and Cd^{2+} concentrations in blood serum of patients with chronic C are 1.187 and 0.00257 ppm, respectively, while in patients with chronic B it were 1.3821 and 0.00381 ppm for Cd^{2+} and Cd^{2+}, respectively. Healthy control donors from the studied areas exhibit different concentrations of the studied ultra trace Cu^{2+} and Cd^{2+} in their serum (0.9504 and 0.00178 ppm, respectively). The results of the studied Cu^{2+} and Cd^{+2} in patient's serum and healthy control are discussed as follows.

Cadmium

Table 3 describes the levels of blood $Cd^{2+}$for the total samples. The results show significant differences of Cd^{2+} concentration between the blood serum of healthy and hepatitis patients. The mean serum Cd^{2+} concentration of patients with chronic C (0.00257 ppm) and patients with chronic B (0.00381ppm) are significantly ($p<0.01$) higher than Cd^{2+}in healthy control (0.00178 ppm).

The level of Cd^{2+}in the human body has a great toxicological significance being responsible for a number of health impairments. In particular, $Cd^{2+}$by binding metallothioneins accumulates mainly in kidney and liver, but also in muscle, bones and skin. It causes renal and kidney dysfunction, and negative effects on the skeleton and on hemopoietic and cardiovascular systems [37], it is also considered as a carcinogenic and genotoxic agent [38]. Cadmium emissions to the environment from mining and non-ferrous smelters are considerable, and additional amounts of Cd pollute the soil from the use of Cd(II) containing sewage sludge and phosphate fertilizers [39]. In addition, Cd^{2+} is contained in cigarettes at level of 1 – 2 µg and approximately the 10% of which is inhaled and the 5% is absorbed [40- 42] . The food categories which mostly account for Cd intake are vegetables and cereals [43-45]. Lead is able to adversely affect the heme-biosynthesis, and gastrointestinal, nervous and cardiovascular systems [46]. The reference values (RVs) for blood $Cd^{2+}$of 215 adult subjects non-occupationally exposed were assessed. Age, sex, smoking, alcohol drinking and living area were used to stratify the reference group [47]. The RVs expressed as 5th – 95th percentiles (geometric mean, GM) were 0.24 – 1.82 μg/l (0.53μg/L) for blood Cd^{2+}. Females had GM levels of Cd^{2+} (0.58μg/L) higher than males (0.49μg/L); subjects aged < 40 years had less Cd^{2+} (0.44μg/L) than old subjects (>60 years; 0.56μg/L); Cd^{2+}in smokers (1.23μg/L) was 3-times higher than in non-smokers (0.42μg/L) and correlated with the number of cigarettes per day. The alcohol intake and place of living did not influence blood Cd^{2+}.

Copper

The mean serum copper level of patients with chronic C (1.187 ppm) and patients with chronic B (1.3821 ppm) are significantly ($p<0.01$) higher than in healthy control (0.950 ppm) as shown in Table 3. The elevated serum copper levels might results from inflammatory responses, it had reported by Kalkan et al. [48] whom studied serum trace elements, including copper in sera of patients with viral hepatitis (A, B, C, D, E) cases and the controls. They have shown elevation in copper levels and have suggested that this probably resulted from defense strategies of organism and induced by hormone – like substances. Copper accumulation in fibrotic livers caused chronic HCV infection may contribute to hepatic injury [49]. Their results shown that the mean serum copper is significantly increased ($P < 0.01$) in patients hepatitis. This elevated may be caused from necrosis of liver parenchyma, leading to an important release of hepatic copper, or decreased hepatobiliary excretions where copper is principally excreted into the bile. Hatono et al. [11] have shown that the hepatic copper contents increased with the progression of hepatic fibrosis and concluded that the presence of copper may enhance HCV infection. Cesur et al. [50] also reported different trend than in our study, they found that serum copper concentration was 103.17 ± 20.8 μg/dl in patients with chronic hepatitis C and 90.8 ± 14.3 μg/dl in healthy subjects. Serum copper concentration was not found statistically different in patients with chronic hepatitis C compared with those of healthy individuals ($P > 0.05$). The authors suggested that changes in liver cell were associated with the functional impairment, which may alter the metabolism of trace elements, in particular copper. Copper is heavily involved in the production of hemoglobin. It is also involved in the production of collagen, the protein responsible for the integrity of bone, catilege, skin and tendon, and elastin, a major component of large blood vessels.

Metal concentrations in hepatitis and healthy blood serum related to areas

Environmental ecosystem may affect the heavy metal contents in blood serum of hepatitis patients, and of healthy ones. Living site is one of environmental ecosystem components that affect the heavy metal contents in their blood. The following results represented the relationship between blood serum heavy metals in hepatitis patients, and healthy controls in the different study areas (Arar, Al-auiqilh, Rafhaa Al-Jouf , Tarif and Al-Qurayyat).

Copper:

Significantly ($p<0.01$) high concentration (1.727±0.4251 ppm) of copper is seen in serum of hepatitis patients from Tarif city than in the other areas, while healthy control from the same city (1.257±0.367 ppm) reveals significantly ($p<0.01$) highest serum Cu^{2+} level (Table 3). The increase of Cu^{2+} concentration in patients from Tarif city may be related to excess Cu^{2+} in agriculture food which surrounding the Fertilizer factory in Tarif or food contamination with Cu^{2+} to the residents. The presence of Cu^{2+} at the highest level in healthy control blood serum of Tarif city may be related to air and water pollution input from industrial activities in this area. Cesur et al. [50] found that serum copper concentration was 103.17±20.8ng/dl in patients with chronic hepatitis C and 90.8±14.3ng/dl in healthy subjects. Serum copper concentration was not found statistically different in patients with chronic hepatitis C compared with those of healthy individuals ($P > 0.05$).

Copper is essential for a variety of biochemical processes and is needed for certain critical enzymes to function in the body. It is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as zinc and molybdenum, and other body functions. The main source of copper is through diet and is present in mineral-rich foods like vegetables, legumes, nuts, grains, fruits, and chocolate. Although normally bound to proteins, copper may be released and become free to catalyze the formation of highly reactive hydroxyl radicals that have a capacity to initiate oxidative damage and interfere with important cellular events. Zinc removes copper from its binding site, where it may cause free radical by Gaetke et el [51].

Copper deficiency caused anemia, growth retardation, defective keratinization and pigmentation of hair, hypothermia, scurvy-like changes in skeleton. Symptoms and diseases of copper toxicity is acne, allergies, alopecia, insomnia, nausea, tooth decay, strokes, yeast infections, urinary tract infections, mood swings, kidney disorders, depression, cystic fibrosis, arthritis, anxiety, anorexia, multiple sclerosis, inflammation, pancreatic dysfunction, vitamin deficiencies, paranoia, migraines, libido decreased, nervousness, osteoporosis, senility, stuttering, phobias, diabetes, autism, autism symptom elevation [52].

Cadmium:

The highest cadmium concentration (0.0057±0.00062ppm) was presented in the serum of hepatitis patients from Al-Qurayyat City (polluted area), while for healthy control, the highest level (0.0023±0.0006 ppm) was observed in those from Tarif city (polluted area). The presence of Cd^{+2} significantly ($p<0.01$) in higher concentrations in donor's serum from Al-Qurayyat and Tarif areas than in those from the other studied areas may be as a result of pollution input from the industrial activities of Detergent factory at Al-Qurayyat, as well as from fertilizer and cement factories at Tarif (Table 3). Rashed [53] reported that among the different hepatitis, patients with hepatitis C virus (HCV) show the highest level of Cd, Cu and Mn in their blood serum, while hepatitis B virus (HBV) show the highest for Co and Pb. Serum trace elements, especially Fe, Zn and Cu, are good marker for diagnosing of liver disease. Hepatitis virus (HAV, HBV and HCV) activities were influenced by the increased or decreased of trace elements.

In conclusion overall results, hepatitis patients from Tarif and Al-Qurayyat cities exhibited significantly ($p<0.01$) higher values of Cu^{2+} and Cd^{+2} in their blood serum than in those from the other areas. Healthy control from Tarif city exhibited higher values of Cu^{2+} and Cd^{+2} in their blood serum than in those from the other areas. Hepatitis patients and healthy control from Arar are a exhibited the lowest values of studied heavy metals (expect Cadmium Rafahaa City) in their blood serum, which reveals that Arar environment is free from pollution with heavy metals. Cu^{2+} and Cd^{+2} contaminations may be included in food. Blood serum heavy metal concentrations differ according to several factors; one of them is the living location of the subject, which differs from one country to another and from one city to another in the same country.

Concentration of Cu^{2+} and Cd^{+2} in blood serum of hepatitis patients related to virus types

Trace elements copper, manganese, selenium and zinc act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects [54]. A delicate balance also exists for the redox trace elements such as copper, which can initiate free radical reactions but is also a cofactor of copper/zinc-superoxide dismutase, a free radical scavenging enzyme. Abundant evidence demonstrates the antioxidant role of copper [55].

Results of Cu^{2+} and Cd^{+2} concentrations in the serum of different hepatitis types (C and B) differed from one virus type to another (Table 3). Patients with hepatitis B virus showed higher levels of Cu^{2+} and Cd^{+2} (1.187 and 0.0038 ppm , respectively) in their serum than those with hepatitis C (1.187and 0.0.0025 ppm, respectively). Lin et.al [56] examined the status of trace elements (selenium, iron, copper, and zinc) in the serum of patients having hepatocellular carcinoma. They observed significant changes in the selenium, iron, copper, and zinc levels relative to those of healthy controls ($p<0.05$). In addition, their finding simply that the levels of some trace elements, such as selenium, iron, copper, and zinc, might serve as biomarkers for the increased severity of viral hepatic damage.

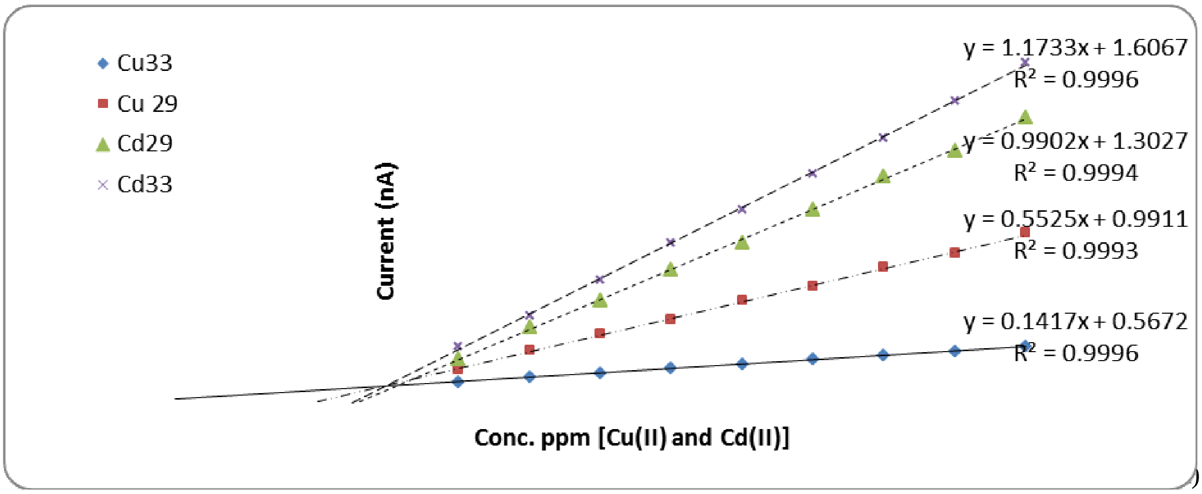
Analytical results

For the analysis of trace amount of heavy metals; most of the analytical multi-element techniques used are inductively coupled plasma-atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS), and stripping voltammetry (SV). For ultra trace analysis, ppb and sub-ppb concentration adsorptive stripping voltammetries (A_dSV) were applied, which make use of electrolytic accumulation of the metal at the stationary electrode surface, mainly hanging mercury drop electrodes (HMDE) on solid electrodes, e.g., glassy carbon is based on adsorptive accumulation of the species on the electrode. This technique has been developed for various cations, anions, and organic molecules [57].

Copper and cadmium concentrations were determined by differential pulse adsorptive stripping voltammetry (DPA_dSV) after complexing with luminol concentration of $1x10^{-4}$ M. Fig. 1 shows the DPA_dSV voltammograms for determination of Cu^{2+} and Cd^{+2} simultaneously by addition of standard copper and cadmium nitrate solutions to the sample in the same electrolysis cell using micropipettes.

Linearity Test of Calibration Plots

Linearity between metal concentration and current was tested for Cu^{2+} and Cd^{+2} by the standard addition methods, and under their optimal conditions. Fig. 2 shows the plots of peak current against concentration of Cu^{2+} and Cd^{+2}. The current of the metal peak increased linearly with standard additions, with a slope similar to that of the supporting electrolyte. Linearity is valid for the studied metals through long periods within the concentration levels normally found in blood serum. The formation of intermetallic compounds between the investigated metal ions may cause an error in their determination. The standard addition method was chosen here because the matrix effects were less influenced by this procedure



) at accumulation potential -0.30V using DPA$_d$SV

Comparison of the analytical methods

A comparative study was carried out between the results of Cu^{2+} and Cd^{+2} concentrations in both patient and healthy blood serums obtained using inductively coupled plasma-atomic emission spectrometry (ICP-AES) and differential pulse adsorptive stripping voltammetry DPA$_d$SV (Table 4).

Table 4 Heavy metal concentrations (ppm) in blood serum of patients related to hepatitis types.

Item Metal		HCV (n=20)		HBV (n=40)		Healthy(n=60)
		ICP-AES	DPA$_d$SV	ICP-AES	DPA$_d$SV	ICP-AES
Cu(II)	Mean	1.1262	1.0994	1.0105	0.9980	Mean ± SD of Total = 0.9527±0.208
	SD	0.7432	0.7252	0.6108	0.6412	Standard error of the mean (SE)=0.02685
	SE	0.1661	0.1621	0.0965	0.1013	Minimum = 0.7896ppm
Cd(II)	Mean	0.002	0.0019	0.0028	0.0026	Maximum= 1.2709ppm
	SD	0.0022	0.0021	0.0064	0.0062	
	SE	0.0005	0.0004	0.0010	0.0009	

It was proved that the results obtained using inductively coupled plasma-atomic emission spectrometry (ICP-AES) for Cu^{2+} (1.126 and 1.0105 ppm) and Cd^{+2} (0,002, and 0.0028ppm, respectively) in blood serum of hepatitis patient virus C and B are nearly in agreement with those obtained using stripping voltammetry of the same elements (1.0994 and 0,9980ppm) for Cu^{2+} and (0.0019 and 0.0026ppm, for Cd^{2+}, respectively).

For healthy control the results obtained using inductively coupled plasma-atomic emission spectrometry (ICP-AES) indicate for Cu, and Cd (1.00349±0.2178, and 0.001936±0.000275ppm, respectively) in blood serum are relatively in agreement with those obtained using stripping voltammetry of the same elements (0.9504±0.1731, and 0.00178±0.00030 ppm, respectively).Generally, the data obtained by inductively coupled plasma-atomic emission spectrometry (ICP-AES) are in close agreement with those obtained by stripping voltammetry for some metals and slight differences for the others.

However, the slight differences that may be found sometimes between both techniques are mainly due to the manipulation of the analyst and metal interferences in cases of inductively coupled plasma-atomic emission spectrometry (ICP-AES) while the standard addition method is used to perform the stripping voltammetry technique. The standard addition method is more accurate than the calibration curves, since additions of the standard analyst to the sample give precise results and minimize or even avoid the interferences usually inherent with the matrix analysis [58].

Effects of Foreign Ions

In the determination of $1x10^{-7}$ $molL^{-1}$ of copper (II) and cadmium (II) at the optimum reagent concentrations studied, foreign ions could be tolerated (less than a 5% change in peak current) at the levels given in Table 2. The great selectivity of the method should be emphasized because only cyanide interferes seriously.

Several metals have been reported to be present in serum samples; cyanide is the most abundant one. Therefore, the effect that this metal produces over the copper and cadmium determination were studied. To detect the possible interference from cyanide, samples of $1x10^{-7}$ $mol.L^{-1}$ of (Cu(II) an Cd(II)) and with different amounts of cyanide were prepared. Ratio (w/w) Cu:CN and Cd: CN were varied from 1:1 to 1:10. The voltammetric signal obtained for Cu(II) and Cd(II) were compared with that corresponding to a sample of this ion, without Cyanide. Signals were considered affected by the cyanide if the difference (1:10) was 5% at least, and it was found that CN did not interfere in the concentration range tested. The determination of copper and cadmium were performed, following the method proposed in this paper. On the other hand, serum samples were also analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). In conclusion, the method of copper and cadmium determination by differential pulse cathodic adsorptive stripping voltammetry this paper is a very low-cost method because reagents and techniques are not expensive. Another advantage is that the time required to carry out the analysis is shorter than other polarographic methods in which determination of copper and cadmium in serum samples was made. It should be pointed out that with this method it is possible to determine very low quantities of copper and cadmium because the detection limits are 0.038 and 0.014 ng ml^{-1}.

Application

The proposed method was successfully applied for determination of ultra trace concentration of copper and cadmium in several serum samples. The Cu^{2+} and Cd^{2+} concentrations of serum were determined using the recommended procedure under optimum conditions, using the standard addition method. The results presented in Table 2, show the high sensitivity of the proposed method.

Chronic hepatitis classification

An input Chronic hepatitis features vector x generates a scalar output $f(x)$ whose polarity – sign of $f(x)$ determines class membership. The magnitude $\|f(x)\|$ can usually be interpreted as a measure of belief or certainty in the decision made. Nearly all binary classifiers can be viewed in these terms; for density based classifiers (Linear, Quadratic and Fisher) the output function f(x) is a log likelihood ratio, whereas for kernel based classifiers (Nearest-Neighbor, RBFs and SVMs) the output is a "potential field" related to the distance from the separating boundary. We will now briefly review the details of the various classifiers used in our study.

Fisher Linear Discriminant

Fisher Linear Discriminant (FLD) is an example of a class specific subspace method that finds the optimal linear projection for classification. Rather than finding a projection that maximizes the projected variance as in principal component analysis, FLD determines a projection, $y = W_F^T x$, that maximizes the ratio between the between class scatter and the within-class scatter. Consequently, classification is simplified in the projected space. Consider a c-class problem, with the between-class scatter matrix given by

$$S_B = \sum_{i=1}^{c} N_i (\mu_i - \mu)(\mu_i - \mu)^T$$

and the within-class scatter matrix by

$$S_W = \sum_{i=1}^{c} \sum_{X_k \in X_i} X_k - \mu_i)(X_k - \mu_i))^T$$

where μ is the mean of all samples, μ_i is the mean of class i, and N_i is the number of samples in class i. The optimal projection W_F is the projection matrix which maximizes the ratio of the determinant of the between-class scatter to the determinant of the within-class scatter of the projections

$$W_F = arg\,\underset{W}{max} \frac{|W^T S_B W|}{|W^T S_W W|} = [w_1 w_2 w_3 \ldots . w_m]$$

where $\{w_i | i = 1,2,\ldots,m\}$ is the set of generalized eigenvectors of S_B and S_W, corresponding to the m largest generalized eigenvalues $\{\lambda_i | i = 1,2,\ldots,m\}$. However, the rank of S_B is c - 1 or less since it is the sum of c matrices of rank one or less. Thus, the upper bound on m is c -1. To avoid the singularity, one can apply PCA first to reduce the dimension of the feature space to N - c, and then use FLD to reduce the dimension to c - 1. This two-step procedure is used in computing "Fisher Faces" [60].

Linear and Quadratic Classifiers

The decision boundary of a quadratic classifier is defined by a quadratic form in x, derived through Bayesian error minimization. Assuming that the distribution of each class is Gaussian, the classifier output is given by

$$\begin{aligned} f(x) = \quad & 0.5(x - \mu_1)^T \sum\nolimits_1^{-1} (x - \mu_1) - \\ & 0.5(x - \mu_2)^T \sum\nolimits_2^{-1} (x - \mu_2) + 0.5\, ln \frac{|\Sigma_1|}{|\Sigma_2|} \end{aligned}$$

where μ_i and $\sum i$ ($i = 1; 2$) are the mean and covariance matrix of the respective Gaussian distributions. A linear classifier is a special case of the quadratic form, based on the assumption that $\sum 1 = \sum 2 = \sum$, which simplifies the discriminant for both classifiers, the sign of $f(x)$ determines class membership and is also equivalent to a likelihood ratio test.

$$f(x) = (\mu_2 - \mu_1)\Sigma^{-1} x + 0.5(\mu_1^T \Sigma^{-1} \mu_1 - \mu_2^T \Sigma^{-1} \mu_2)$$

Gender Classification by SVM

Support vector machines (SVMs) are classifiers that construct a maximal separating hyper plane between two classes so that the classification error is minimized. For linearly no separable data the input is mapped to high-dimensional feature space where they can be separated by a hyper plane. This projection into high-dimensional feature space is efficiently performed by using kernels. For instance-label pair (x_i, y_i) with $x_i \in \mathcal{R}^n, y_i \in \{1 - ,1\}$ for $1 \leq i \leq n$ where n is the number of instances, the following optimization problem needs to be solved for SVMs

$$\min_{w,b,\xi} \frac{1}{2} w^T w + C \sum_{i=1}^{n} \xi_i$$

Subject to

$$y_i(w^T \phi(x_i) + b0 \geq 1 - \xi_i \ , \ \xi_i \geq 0$$

In the above equation, C is the penalty parameter for error term and ϕ maps training Instance x_i to higher dimensional space. The kernel K is defined as:

$$K = (x_i, x_j) = \emptyset(x_i)^T \emptyset(x_j)$$

In this paper, a Radial Basis Function (RBF) kernel was used which is defined as:

$$K = (x_i, x_j) = exp(-\gamma \|x_i - x_j\|^2 \ , \ \gamma \geq 0$$

The parameter γ controls the spread of a Gaussian cluster. Therefore in the above formulation there are two parameters, C and γ to control the performance of the classifier. Table 3show the comparison between SVM and other classifiers techniques

Table 5: comparison between SVM and other classifiers techniques

Classifier	Error Rate HVB			Error Rate HVC		
	Overall	Male	Female	Overall	Male	Female
SVM	9.49 %	9.04 %	11.43 %	10.19 %	8.29 %	8.43 %
FLD	60.35 %	49.38 %	52.24 %	50.69 %	46.35 %	39.13 %
Quadratic	42. 85%	36.72 %	44.83 %	40. 89 %	32.11 %	46.87 %

Conclusion

This study clearly showed that a simple model consisting of clinical data with SVM could identify CHC patients with interferon treatment efficacy (effective group and ineffective group) with a higher degree of accuracy. Thus, the application of this model can be a useful reference for doctors when making decisions regarding interferon treatment. And also this studied demonstrated that the adsorptive stripping analysis of copper and cadmium in the presence of luminol is an excellent method for the determination of ultra trace concentrations of these cations. In conclusion, the above system offered a practical potential for the simultaneous determination of copper and cadmium; especially having advantages of high sensitivity, high selectivity, simplicity and speed. Thus, this study showed that concentration of blood serum for both hepatitis patients differ according to virus type and living sites. Copper and cadmium presented at higher level in patient serum than in healthy ones, and also Cu(II) and Cd(II) concentration presented at lower levels in patient virus C serum than in patient virus B serum ones, indicating that these metals have a role in virus activity. Therefore, the importance of considering, identifying, and removing metals, if present in excessive amounts, is essential for maximizing hepatitis B treatments. The results obtained for the blood samples by (DPA_dSV) and (ICP-AES) are in very good agreement, and show that both are suitable for the determination of Cu(II), and Cd(II) in blood samples at micro-levels.

Acknowledgment

The authors wish to thank physicians and all staff members of pathogenesis of Arar educational, Prince Abdul Aziz bin mosaed, Central Rafhaa, Prince Abdul Rahman Al-Sudairi Jouf and Al- Qurayyat hospital for their assistance and kindness during the collection of patient blood samples, clinical analysis, diagnosis of hepatitis, and assistance in interpretation of clinical data.

References

[1] Jun Yang · Anto Satriyo Nugroho · Kazunobu Yamauchi · Kentaro Yoshioka ·Jiang Zheng · Kai Wang · Ken Kato · Susumu Kuroyanagi · Akira IwataEfficacy of Interferon Treatment for Chronic Hepatitis C Predicted by Feature Subset Selection and Support Vector Machine. J Med Syst (2007) 31:117–123

[2] Rashed MN, Mahasen Mohamed Ahmed, Ahmed Farouk Al-Hossainy, Safaa Mahmoud Abd . Trends in speciation analysis of some heavy metals in serum of patients with chronic hepatitis C and chronic hepatitis B using differential pulse adsorptive stripping voltammetric measurement and atomic absorption spectrophotometry. Journal of Trace Elements in Medicine and Biology 24 (2010) 138–145

[3] Mortada WI, Sobh MA, El-Defrawy MM, Farahat SE, Reference intervals of cadmium, lead, and mercury in blood, urine, hair, and nails among residents in Mansoura city, Nile delta, Egypt. Environ. Res. 2002; 90: 104.

[4] Reinhold JG. Trace elements: a selective survey. Clin Chem 1975;21:476–500.

[5] Delves HT. Assessment of trace element status. Clin Endocrinol Metab 1985;14:725–760.

[6] Papageorgiou T, Zacharoulis D, Xenos D, Androulakis G. Determination of trace elements (Cu, Zn, Mn, Pb) and magnesium by atomical absorption in patients receiving total parenteral nutrition. Nutrition 2002; 18:32–34.

[7] Guanghan, L., Dewu, L., Dehua, L., Tong, Z., Hongyan, Z., & Chuanyan, L. (2004). Determination of copper in food by stripping voltammetry using cu(II)-[2-(2,3,5-triazolylazo)-5-dimethulanino-benzoic acid] complex. Food Chemistry, 84,319–322.

[8] Gholivand, M. B., Sohrabi, A., & Abbasi, S. (2007). Determination of copper by adsorptive stripping voltammetry in the presence of calcein blue. Electroanalysis, 19, 1609–1615.

[9] Adinarayan Reddy, S., Janardhan Reddy, K., Lakshmi Narayan, S., & Varada Reddy, A. (2008). Analytical application of 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone and determination of cu(II) in food samples. FoodChemistry, 109, 654–659.

[10] Shams, E., Babaei, A., & Soltaninezhad, M. (2004). Simultaneous determination of copper, zinc and lead by adsorptive stripping voltammetry in the presence of morin. Analytica Chimica Acta, 501, 119–124.

[11] Tanasescu, C.; Baldescu, R. and Chirulescu, Z. (1996): “Interdependence between Zn and Cu serum concentrations and serum immunoglobulins in liver diseases” Rom. J. Int. Med., 34: 217 – 224.

[12] Hatono, R.; Ebara, M.; Fukuda, H.; Yoshikawa, M.; Sugiura, N.; Kondo, F.; Yukawa, M. and Saisho, H. (2000) “Accumulation of copper in the liver and hepatic injury in chronic hepatitis C”. J. Gastroenterol. Hepatol., 15(7): 786 – 791.

[13] Kalkan, A, Bulut, V.; Avci, S.; Celik, I. And Bingol, N.K. (2002): “Trace elements in viral hepatitis”. J. Trace Elem. Med Biol. 16 pp: 227 – 230.

[14] Kakkar P, Jaffery FN, biological markers for metal toxicity. Environmental Toxicology and Pharmacology, Environ Tox Pharmacol, 2005, 19: 335.

[15] Boffetta P, Mutat Res, Human cancer from environmental pollutants: the epidemiological evidence, Genet. Toxicol. Environ. Mutagen., 2006, 608: 157

[16] Fábio Kummrow, Fábio F. Silva, Rúbia Kuno, Alexandre L. Souza, Pedro V. Oliveira. Biomonitoring method for the simultaneous determination of cadmium and lead in whole blood by electrothermal atomic absorption spectrometry for assessment of environmental exposure. Talanta 75 (2008) 246–252

[17] Roberts, J.R.: Metal toxicity in children. In training manual on pediatric environmental health: putting it into practice 1999 Jun. Emeryville, CA: children’s Environmental Health Network (http://www.Cehn. org/cehn/trainingmanual/pdf/manualfull.pbf).

[18] Mahajan RK, Walia TP, Sumanjit Lobana TS. Cyclopen-tanone thiosemicarba- zone, a new complexing agent for copper determination in biological samples by adsorptive stripping voltammetry. Anal Sci 2006;22(3):389–92.

[19] Kumar P, Mouli PC, Reddy SJ, Mohan SV. Differential pulse anodic stripping voltammetric determination of Pb, Cd, Cu, and Zn in air, diet, and blood samples: exposure assessment. Anal Lett 2005;38(3):463–75.

[20] Templeton DM. Quantifying cadmium and lead in whole blood. Clin Chem 1992;38(10):1927–9.

[21] Tripathi RM, Raghunath R, Mahapatra S, Sadasivan S. Blood lead and its effect on Cd, Cu, Zn, Fe and hemoglobin levels of children. Sci Total Environ 2001;277(1–3):161–8.

[22] Zhang ZQ, Liu H, Zhang H, Li YF. Simultaneous cathodic stripping voltam- metric determination of mercury, cobalt, nickel and palladium by mixed binder carbon paste electrode containing dimethylglyoxime. Anal Chim Acta 1996;333:119–24.

[23]Jyothi Y, Sreedhar NY. Extraction-stripping voltammetric determination of copper, cobalt and nickel in human serum. Bull Electrochem 2005;21(5): 209–11.

[24] Vasilescu, M., Constantinescu, T., Voicescu, M., Lemmetyinen, H., & Vuorimaa, E. (2003). Spectrophotometric study of luminol in dimethyl sulfoxide–potassium hydroxide. Journal of Fluorescence, 13, 315–322.

[25] Abbasi, S., Farmany, A., Gholivand, M. M., Naghipour, A., Abbasi, F., & Khani, H. (2009). Kinetic-spectrophotometry method for determination of ultra trace of aluminum in food samples. Food Chemistry, 116, 1019–1023

[26] Laux, Dale L., Law Enforcement Technology, May, 26-27 (1991).

[27] Golcu, A., Tume, M., Demirell, H., & Wheatley, R. A. (2005). Cd(II) and Cu(II) complexes of polydentate schiff base ligands: synthesis, characterization, properties and biological activity. Inorganica Chimica Acta, 358, 1785–1797.

[28] Gholivand, M. B., & Romiani, A. A. (2006). Application of adsorptive stripping voltammetry to the simultaneous determination of bismuth and copper in the presence of nuclear fast red. Analytica Chimica Acta, 571, 99–104.

[29] Ahmed Farouk Al-Hossainy, Determination of Copper with 5-(dimethylamino)-2-((4-methyl-4H-1,2,4-triazol-3-yl)diazenyl) benzoic acid in Water Samples of River Nile (Egypt) by Adsorptive Stripping Square Wave Voltammetry . Egypt J. Anal. Chem., Vol. 19,PP. 52-66 (2010)

[30] Beltagi, A. M., & Ghoneim, M. M. (2009). Simultaneous determination of trace aluminium, copper and cadmium in water samples by square-wave adsorptive cathodic stripping voltammetry in the presence of oxine. Journal of Applied Electrochemistry, 39, 627–636.

[31] Sancho, D., Deban, L., Pardo, R., & Valladolid, D. (2005). Determination of zinc, cadmium, lead and copper in pellets and pulp of sugar beet. Journal of the Science of Food and Agriculture, 85, 1021–1025.

[32] Babaei, A., Babazadeh, M., & Shams, E. (2007). Simultaneous determination of iron, copper and cadmium by adsorptive stripping voltammetry in the presence of thymolphthaloxene. Electroanalysis, 19,9, 978–985.

[33] Iliadou, E. N., Girousi, S. T., Dietze, U., Voulgaropoulos, A. N., & Papadopoulos, C. G. (1997). Simultaneous determination of nickel, cobalt, cadmium, lead and copper by adsorptive voltammetry using 1-phenylpropane-1- pentylsulfonylhydrazone-2-oxime as a chelating agent. Analyst, 122, 597–600.

[34] Jakmunee, J., & Junsomboon, J. (2008). Determination of cadmium, lead, copper and zinc in the acetic acid extract of glazed ceramic surfaces by anodic stripping voltammetric method. Talanta, 77, 172–175.

[35] Ensafi, A. A., Khayamian, T., Benvidi, A., & Mirmomtaz, E. (2006). Simultaneous determination of copper, lead and cadmium by cathodic adsorptive stripping voltammetry using artificial neural network. Analytica Chimica Acta, 561, 225–232.

[36] Long GL. Winefordner JD. Limit of detection: A closer look at the IUPAC definition. Anal., Chem.,1983. 55, 713A

[37] FAO/WHO, 2001. Cadmium. Safety Evaluation of Certain Food Additives and Contaminants. WHO Food Additive Series, vol. 46. FAO/WHO, Geneva, Switzerland.

[38] IARC, 1997. Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 58. Lyon, France.

[39] Van Assche, F.J., 1998. A stepwise model to quantify the relative contribution of different environmental sources to human cadmium exposure. In: Proceedings of the 8th International Nickel–Cadmium Battery Conference, Prague, Czech Republic, September 21–22.

[40] Elinder, C.-G., Kjellstrom, T., Lind, B., Linnman, L., Piscator, M., Sundstedt, K., 1983. Cadmium exposure from smoking cigarettes: variations with time and country where purchased. Environ. Res. 32, 220–227.

[41] Friberg, L., Kjellstrom, T., Nordberg, G.F., 1986. Cadmium. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds.), Handbook on the Toxicology of Metals. Elsevier, Amsterdam, pp. 130–184.

[42] WHO, 1992. International Programme on Chemical Safety. Environmental Health Criteria 134. Cadmium.World Health Organization, Geneva.

[43] Dabeka, R.W., McKenzie, A.D., 1995. Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986–1988. J. AOAC Int. 78, 897–909.

[44] Tripathi, R.M., Raghunath, R., Krishnamoorthy, T.M., 1997. Dietary intake of heavy metals in Bombay city, India. Sci. Total Environ. 208, 149–159

[45] Larsen, E.H., Andersen, N.L., Moller, A., Petersen, A., Mortensen, G.K., Petersen, J., 2002. Monitoring the content and intake of trace elements from food in Denmark. Food Addit. Contam. 19, 33–46.

[46] FAO/WHO, 2001. Cadmium. Safety Evaluation of Certain Food Additives and Contaminants. WHO Food Additive Series, vol. 46. FAO/WHO, Geneva, Switzerland.

[47] Giovanni Fortea, Roberto Madeddub, Paola Tolub, Yolande Asarab, Juan A. Marchald, Beatrice Boccaa. Reference intervals for blood Cd and Pb in the general population of Sardinia (Italy). International Journal of Hygiene and Environmental Health 214 (2011) 102–109

[48] Kalkan, A.; Bulut, V.; Avci, S.; Celik, I. And Bingol, N.K. (2002: "Trace elements in viral hepatitis". J. Trace Elem. Med Biol. 16 pp: 227 – 230.

[49] Chaturvedi UC, Shrivastava R, Upreti RK. Viral infections and trace elements: a complex interaction. Curr Sci 2004;87(11):1536–54.

[50] Cesur S, Cebeci SA, Kavas GO, Aksaray S, Tezeren D. (Serum copper and zinc concentrations in patients with chronic hepatitis B. J Infect 2005;51:38–40.

[51] Gaetke LM, Chow CK. Copper toxicity oxidative stress and antioxidant nutrients. Toxicology 2003;189:147–63.

[52] Elihu N, Chelation Therapy in Cardiovascular Disease: Ethylenediaminetetraacetic Acid, Deferoxamine, Dexrazoxane." Journal Clinical Pharmacology. 1998. 38, 101-105.

[53] Mohamed Nageeb Rashed, The role of trace elements on hepatitis virus infections: A review. Journal of Trace Elements in Medicine and Biology. 25 (2011) 181– 187.

[54] Rukgauer M, Neugebauer RJ, Plecko T. The relation between selenium, zinc and copper concentration and the trace element dependent antioxidative status. J Trace Elem Med Biol 2001;15:73–8.

[55] Konukoglu D, Ercan M, Ayaz M, Onen S. Plasma and erythrocytes antioxidant status and trace element level in proteinuric patients with moderate glomerular function. J Trace Elem Med Biol 2001;15:119–22.

[56]] Lin C. C, Huang J. F, Tsai L. Y, Huang Y. L. Selenium, iron, copper, and zinc levels and copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem Res 2006;109(1):15–24.

[57] Farghaly OA. Direct and simultaneous voltammetric analysis of heavy metals in tap water samples at Assiut city: an approach to improve the analysis time for nickel and cobalt determination at mercury film electrode. Microchem J 2003;75(2):119–31.

[58] Eaton, AD, Clesceri, LS, Greenberg, AE. Standard methods for the examination of water and waste water. USA, 1995.

[60] Sareni, B., Krahenbuhl, L., & Nicolas, A." Efficient genetic algorithms for solving hard constrained optimization problems", IEEE Transaction on Magnetics, 36(4), pp.1027–1030.2000.

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/journals/ The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar