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ANALYSIS OF HUMAN SERUM WITH VARIOUS CATARACTS BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY BY USING DIFFERENT DIGESTION PROCEDURES

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ABSTRACT

Three digestion procedures are evaluated for the determination of essential elements such as Co, Ni, Mn, Fe, Cr, Zn, Cu, Cd, Pb, Mo and V using different acid mixtures. The concentration of Fe, Co, Ni, Mn, Cr, Cu, Cd, Pb, Mo and V in the serum of cataract patients was also determined by using Inductively coupled plasma atomic emission spectroscopy (ICP – AES) technique in an effort to evaluate the status of these elements in such patients and to further clarify their role in cataract disease. Various parameters such as variation of acids, ratio of acid mixture, effect of temperature and effect of time were checked for optimization. The 3 digestion procedures include wet digestion with $\text{HNO}_3 - \text{H}_2\text{O}_2$, $\text{HNO}_3 - \text{H}_2\text{SO}_4$ and $\text{HNO}_3 - \text{HClO}_4$. Among these, the $\text{HNO}_3 - \text{HClO}_4$ was selected for complete digestion of serum samples.

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Key Words

Serum samples, ICP - AES, Serum digestion, Cataract disease.

INTRODUCTION

The increasing importance of trace elements in human health has been realized over the last two decades. Because of the enhanced mobility of metals in the environment, an increased interest in these elements in living organisms has been observed. A great majority of the trace elements serve chiefly as key components in enzyme systems. Human Serum is an important diagnostic fluid for the study of the pathogenesis of several diseases (For example, an elevated amount of 'Cu' was recently found in the serum of patients with Alzheimer's disease) [1]. Human serum is also extensively used in clinical analysis. Indeed the concentrations of nutritional elements such as Fe, Cu, Zn, Co, Se and Mo must fall within acceptable minimum and maximum standards to endure good health. Inductively coupled plasma mass spectrometry (ICP – MS) is a powerful multi – elemental detection technique with low detection limits, which is being applied to the analysis of a variety of samples including biological materials such as human serum [2,3]. The analysis of serum is nonetheless still challenging because of its complex matrix, which can induce both spectroscopic and non-spectroscopic interferences during the analysis. The most commonly used measure of nutritional status is the level of trace element in blood, either whole blood or some fraction. There are many factors besides nutrition, which affect the levels of these elements including infection, hormones, and pregnancy and sampling time. Representative metals known to induce delayed hypersensitivity include Beryllium, Chromium, Cobalt, Copper, Gold, Manganese, Mercury, Molybdenum, Nickel, Platinum and Zirconium. Hypersensitivity to chromium is one of the more common clinical allergies and is often with a reactivity to Cobalt or Nickel compounds[4,5].

Iron, Cr, Co and Zn are essential trace elements (micronutrients) for living organisms. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells [6]. Iron deficiency is the most prevalent nutritional deficiency in humans [7] and is commonly caused by insufficient dietary intake; Iron is important because it eliminates

phlegm and strengthens the function of stomach. The requirement of iron for an adult is 20 mg / day and for a child is 10 mg/day. Iron is found in the range of 1.5-21 µg ml⁻¹.

Chromium is an essential element since it is a cofactor for insulin and a component of the glucose tolerance factor [8]. Chromium is found in the range of 0.05-0.2 µg/ml. The recommended dietary intake extends from 50 to 250 µg. Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be a risk factor in atherosclerotic disease [9]. Offenbacher and Pi – Sunyer [10], further suggested that chromium uptake is influenced by certain plasma proteins, namely transferrin and albumin, like Iron and Zinc.

Chaoyang Huang and Diane Beauchemin [11] used multielemental analysis of human serum by ICP – MS with on-line standard addition using flow injection. Trace and ultra trace elements in human blood and serum have attracted the great attention and interest in medical and biological sciences for the diagnosis of human health, disease and nutrition [12, 13]. A method is presented by Ali Bazzi et al [14], for the determination of antimony in whole human blood samples with an ICP-MS instrument using a quadrupole mass analyzer. A nitric acid/hydrogen peroxide open digestion procedure was employed for the blood sample treatment and preparation for analysis. The precision and accuracy of the method were evaluated by analyzing several Seronorm trace elements whole blood reference materials. Kimiyoshi Kitamura et al [15] studied the effective pretreatment of human serum samples for dioxin analysis by solid phase extraction and blue – chitin column cleanup. Maria Fernanda Gine et al [16] studied the determination of trace elements in serum samples by isotope dilution inductively coupled plasma mass spectrometry using on-line dialysis. Jorgen Riondato et al [17] determined the trace and ultra trace elements, non-spectrally interfered (Cd, Sn, Ag and U) and the spectrally interfered (Al, Si, P, S, Ti, Cr, Mn, Fe, Cu and Zn) elements by with a double Focusing Magnetic Sector Inductively Coupled Plasma Mass Spectrometer.

Alimonti et al [18] described a method for the determination of 14 trace and ultra trace elements (

Al,Cd,Co,Li,Mn,Mo,Ni,Pb,Pt,Rb,Sb,Se and Zn) in serum of term and pre-term newborns by inductively coupled plasma mass spectrometry (ICP-MS) with ultrasonic nebulization. An algorithmic approach to understand trace elemental homeostasis in serum samples of Parkinson disease by Sanjay Pande [19]. Nese Cokuras et al [20] studied on the effects of Ni²⁺ Co²⁺ and Mn²⁺ on Human serum.

In the present work, Three digestion procedures were evaluated for the determination of essential elements such as Co, Ni, Mn, Fe, Cr, Zn, Cu, Cd, Pb, Mo and V using different acid mixtures. The concentration of Fe, Co, Ni, Mn, Cr, Cu, Cd, Pb, Mo and V in the serum of cataract patients was also determined in an effort to evaluate the status of these elements in such patients and to further clarify their role in this disease. Various parameters such as variation of acids, ratio of acid mixture, effect of temperature and effect of time were checked for optimization. The 3 digestion procedures include wet digestion with HNO₃ – H₂O₂, HNO₃-H₂SO₄ and HNO₃ – HClO₄. Among these, the HNO₃ – HClO₄ was selected for complete digestion of serum samples.

Table 1 JOBIN – YVON Inductively Coupled Plasma Atomic Emission Spectrometer operating conditions.

S.No.	Metal	Wavelength (nm)	S.No.	Metal	Wavelength (nm)
1.	Fe	259.940	7.	Cu	324.754
2.	Co	237.862	8.	Zn	213.856
3.	NI	232.003	9.	Cd	228.802
4.	Mn	257.610	10.	Pb	220.353
5.	Cr	205.559	11.	Mo	281.615
6.	V	310.230			

A. Nebulization System

Jobin –Yvon Crossed flow nebulizer system	
1	Incident power (KW) = 1.0
2	Nebulizer pressure (bar) = 3.0
3	Plamsa gas (l/min) = 16
4	Sample flow rate (ml/min) = 1.7

Collection of Human blood serum samples

Human blood samples were collected from 10 persons, suffering from cataracts with a 10ml polypropylene syringe equipped with silicon – coated glass tubes and

centrifuged at 3000 rpm for 20 min. The supernatants were collected as the blood serum samples.

EXPERIMENTAL

MATERIALS AND METHODS

A JOBIN – YVON PANORAMA Inductively Coupled Plasma Atomic Emission Spectrometer with axial viewing configuration was used for all spectroscopic measurements. The details of operating conditions are listed in Table 1. All reagents used in the present work were of analytical – reagent grade. Suprapure analytical grade nitric acid – 65% (w w-1), hydrogen peroxide – 30% (w w-1) and perchloric acid (69-72%) were used for digestion of serum samples. All glass and plastic ware was thoroughly cleaned and acid washed in 5% (v v-1) nitric acid and then rinsed in deionized water prior to use to avoid metal contamination.

Multi element working standards were prepared containing Co, Ni, Mn, Fe, Cr, Cu, Cd, Pb, Zn, Mo and V by diluting high purity 1000mg L-1 stock solutions with deionized water and nitric acid.

centrifuged at 3000 rpm for 20 min. The supernatants were collected as the blood serum samples.

Digestion procedures

Digestion of serum samples using the acid mixture HNO₃ – H₂O₂

0.5 ml of serum was placed in a beaker and 10 ml of acid mixture HNO₃:H₂O₂ was added and heated on hot plate by varying the factors such as ratio of acid mixture, effect of digestion time and effect of temperature. Among these a 4:2 ratio of HNO₃:H₂O₂ was added and the temperature was fixed at 1200C for 30 min. After

cooling to the room temperature and contents were diluted with double distilled water. For optimization we have used the combinations like 4:1, 4:3 and 3:2 were used with temperature varying between 800C and 1200C and dissolution time was fixed between 15 and 60 minutes.

Digestion of serum samples using the acid mixture: HNO₃–H₂SO₄

0.5 ml of serum was placed in a beaker and 10 ml of acid mixture HNO₃: H₂SO₄ was added and heated on hot plate by varying the factors such as ratio of acid mixture, effect of digestion time and effect of temperature. Among these a 4:2 ratio of HNO₃: H₂SO₄ was added and the temperature was fixed at 1200C for 30 min. After cooling to the room temperature and contents were diluted with double distilled water. For optimization we have used the combinations like 4:1, 4:3 and 3:2 were used with temperature varying between 800C and 1200C and dissolution time was fixed between 15 and 60 minutes.

Digestion of serum samples using the acid mixture: HNO₃ – HClO₄

0.5 ml of serum was initially heated on a hot plate with 10 ml of nitric acid for approximately 30 min at 1100C.

Table .2 Elemental concentrations in serum of cataract persons ($\mu\text{g ml}^{-1}$) using Acid Mixture HNO₃ – H₂O₂

Sr.No	Sex	Age	Disease	Co	Ni	Mn	Fe	Cu	Cd	Pb	Zn	Cr	V	Mo
1	F	35	Mature Cataract	N.D	0.04	0.43	1.58	0.21	N.D	0.44	4.66	N.D	N.D	N.D
2	M	50	Mature Cataract	0.24	0.56	0.39	20.46	1.08	0.13	0.94	2.35	N.D	2.16	0.54
3	F	55	Mature Cataract	N.D	N.D	0.31	3.96	0.84	N.D	0.66	N.D	0.07	1.60	0.46
4	M	45	Mature Cataract	0.26	0.54	0.41	8.90	0.98	0.06	0.85	3.11	0.03	N.D	0.33
5	F	50	Mature Cataract	0.18	0.72	0.34	8.21	N.D	N.D	0.94	2.28	N.D	2.63	0.61
6	F	55	Mature Cataract	0.33	0.74	0.31	11.98	0.95	0.11	1.13	2.19	0.46	3.15	0.55
7	M	55	Brown Cataract	N.D	0.36	0.12	1.02	0.73	N.D	0.45	1.41	N.D	1.58	0.18
8	F	15	Traumatic Cataract	0.11	N.D	0.19	3.53	0.46	N.D	N.D	1.79	N.D	1.14	N.D
9	F	60	Brown Cataract	0.20	N.D	0.36	10.26	1.02	0.14	1.89	6.90	N.D	2.08	0.23
10	M	15	Traumatic Cataract	0.16	0.34	0.23	10.98	N.D	N.D	0.03	6.91	0.26	1.08	0.21

N.D. Not Determined

Heating was supplied to maintain gentle boiling of the solution. The reaction was carried out in a 100 ml beaker covered with watch glass to prevent the loss of the sample. Further, 10 ml of HNO₃ was added and heating continue for a further 30 min. A 2.0 ml of portion of 70% perchloric acid was subsequently added and the contents were gently heated on a hot plate until the solutions became colorless, and white fumes of HClO₄ were evolved. When the solutions were cooled to room temperature, the contents were brought to volume double distilled water and the solutions were mixed with a magnetic stirrer.

Trace elemental determination

All the samples after digestion were subjected to ICP AES analysis.

ICP – AES determination

The main operational conditions of the Inductively Coupled Plasma Atomic Emission Spectrometer are summarized in Table 1. The measurement conditions were standardized signal to background ratio. Analytical results were calculated using the straight calibration graphs based on acidified multi element standard solution. All measurements were repeated three to five times for three individual digests.

Table .3 Elemental concentrations in serum of cataract persons ($\mu\text{g ml}^{-1}$) using Acid Mixture $\text{HNO}_3 - \text{H}_2\text{SO}_4$

Sr. No	Sex	Age	Disease	Co	Ni	Mn	Fe	Cu	Cd	Pb	Zn	Cr	V	Mo
1	F	35	Mature Cataract	N.D	0.05	0.47	1.56	0.21	N.D	N.D	4.71	0.02	N.D	N.D
2	M	50	Mature Cataract	0.40	0.48	0.46	20.86	1.99	0.12	0.92	2.21	0.10	2.26	0.43
3	F	55	Mature Cataract	0.18	0.29	0.28	3.86	1.13	0.06	0.63	2.96	0.08	1.38	0.36
4	M	45	Mature Cataract	0.26	0.36	0.41	9.18	1.01	0.09	0.84	3.16	0.05	0.24	0.42
5	F	50	Mature Cataract	0.35	0.74	0.33	8.52	1.06	N.D	0.96	2.19	0.31	2.66	0.62
6	F	55	Mature Cataract	0.41	0.83	0.36	12.36	1.09	0.12	1.18	2.31	0.46	3.54	0.59
7	M	55	Brown Cataract	0.19	0.41	0.13	1.49	0.69	0.07	0.53	1.49	N.D	N.D	0.26
8	F	15	Traumatic Cataract	N.D	0.26	0.28	3.38	0.48	0.04	0.58	1.89	0.46	1.19	0.16
9	F	60	Brown Cataract	0.26	0.48	0.41	11.42	1.05	0.58	2.35	6.99	0.09	1.86	0.38
10	F	15	Traumatic Cataract	N.D	N.D	0.31	11.23	0.49	0.06	0.45	7.34	0.46	1.39	N.D

RESULT N.D. Not Determined

Usually, the digesting reagent for a digestion system is nitric acid. Hydrogen peroxide generally used on the remaining organic materials. However, low recoveries of aluminum from plants and sea food samples have been observed with HNO_3 or $\text{HNO}_3 - \text{H}_2\text{O}_2$. Twelve elements

in 10 serum samples of various cataracts were determined with 3 digestion procedures [21-23].

Analysis of elemental concentrations in serum of cataract persons was made by adopting different digestion procedures by using ICP-AES. The results of elemental concentrations in serum of cataract persons have been shown in tables 2, 3 and 4.

Table .4 Elemental concentrations in serum of cataract persons ($\mu\text{g ml}^{-1}$) using ACID MIXTURE $\text{HNO}_3 - \text{HClO}_4$

Sr. No.	Sex	Age	Disease	Co	Ni	Mn	Fe	Cu	Cd	Pb	Zn	Cr	V	Mo
1	F	35	Mature Cataract	0.012	0.06	0.50	1.61	0.50	0.04	0.05	4.75	0.05	0.33	N.D
2	M	50	Mature Cataract	0.50	0.97	0.44	27.04	2.33	0.20	1.51	2.61	0.19	4.47	0.92
3	F	55	Mature Cataract	0.29	0.51	0.25	4.01	1.05	0.10	0.94	1.86	0.112	2.10	0.50
4	M	45	Mature Cataract	0.39	0.73	0.43	9.33	1.21	0.14	1.07	3.27	0.10	0.33	0.66
5	F	50	Mature Cataract	0.49	1.06	0.37	8.43	1.39	0.20	1.26	2.43	0.49	3.93	0.79
6	F	55	Mature Cataract	0.55	1.10	0.40	12.22	1.58	0.22	1.70	2.25	0.82	5.0	0.93

7	M	55	Brown Cataract	0.34	0.58	0.14	1.56	0.95	0.10	0.79	1.58	0.05	2.28	0.46
8	F	15	Traumatic Cataract	0.23	0.49	0.26	3.50	0.69	0.09	0.74	1.98	0.75	1.63	0.31
9	F	60	Brown Cataract	0.35	0.62	0.43	11.58	1.70	0.77	3.86	8.20	0.14	2.85	0.53
10	M	15	Traumatic Cataract	0.25	0.46	0.24	3.26	0.65	0.13	0.78	1.88	0.81	1.59	0.42

N.D. Not Determined

In the digestion procedures A and B, a light yellow colour was observed remaining at the end. Extreme care was taken to avoid bumping. The elements Co, Ni, Mn, Cr, Fe, Cu, Cd, Pb, Zn, Mo and V were determined after the complete digestion and shown in Tables 2, 3 and 4.

It is observed from the tables 2, 3 and 4 that the concentration of cobalt is highest in the same sample (No.6) at 0.33 in HNO₃ – H₂O₂ mixture, at 0.41 in HNO₃ – H₂SO₄ mixture and at 0.55 in HNO₃ - HClO₄ where as the concentrations of cobalt were could not be detected in (No.1) HNO₃ – H₂O₂ mixture and HNO₃ – H₂SO₄ mixture and concentration was detected at lowest concentration at 0.012 when HNO₃- HClO₄ mixture was used.

It is noticed from the tables 2, 3 and 4 that the concentration of Nickel is highest in the same sample (No.6) at 0.74 in HNO₃ – H₂O₂ mixture, at 0.83 in HNO₃ – H₂SO₄ mixture and at 1.10 in HNO₃- HClO₄ where as the concentrations of Nickel is lowest in same sample (No.1) at 0.04, 0.05 and 0.06 respectively in HNO₃ – H₂O₂ mixture, HNO₃ – H₂SO₄ mixture and HNO₃ - HClO₄ mixture.

The elemental concentration of Manganese is observed highest at 0.43, 0.47 and 0.50 in HNO₃ – H₂O₂ mixture, HNO₃ – H₂SO₄ mixture and HNO₃ - HClO₄ mixture respectively for the same sample (No.1). The lowest concentration of Manganese is also observed lowest at 0.12, 0.13 and 0.14 in HNO₃ – H₂O₂ mixture, HNO₃ – H₂SO₄ mixture and HNO₃ - HClO₄ mixture respectively in the same sample (No.7)

The elemental concentration of iron is observed highest at 20.46, 20.86 and 27.04 in HNO₃ – H₂O₂ mixture, HNO₃ – H₂SO₄ mixture and HNO₃ - HClO₄ mixture respectively for the same sample (No.2). The lowest

concentration of iron is also observed lowest at 1.02, 1.49 and 1.56 in HNO₃ – H₂O₂ mixture, HNO₃ – H₂SO₄ mixture and HNO₃ - HClO₄ mixture respectively in the same sample (No.7).

The elemental concentration of copper is observed highest at 1.08, 1.99 and 2.33 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.2). The lowest concentration of copper is also observed lowest at 0.21, 0.21 and 0.50 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively in the same sample (No.1)

The elemental concentration of cadmium is observed highest at 0.14, 0.58 and 0.77 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.9). The lowest concentration of cadmium is also observed lowest at 0.04 and 0.09 (sample No. 8) when HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively in the same sample (No.8) the elemental concentration of cadmium could not be detected when HNO₃ – H₂O₂ mixture is used for digestion.

The elemental concentration of lead is observed highest at 1.89, 2.35 and 3.86 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.9). The lowest concentration of lead is also observed lowest at 0.03, 0.45 and 0.78 (sample No. 10) when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively.

The elemental concentration of zinc is observed highest at 6.91, 7.34 and 8.20 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.10). The lowest concentration of zinc is also observed lowest at 1.41, 1.49 and 1.58 (sample No. 7) when HNO₃ – H₂O₂, HNO₃

– H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively.

The elemental concentration of chromium is observed highest at 0.46, 0.46 and 0.82 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.6). The same value of 0.46 as highest is observed in more than one sample when HNO₃ – H₂SO₄ mixture was used. It indicates that the samples belong to either same area or exposed to similar occupation. The lowest concentration of chromium is also observed at 0.02 and 0.05 (sample No. 1) when HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively. Elemental concentration of chromium could not be detected when HNO₃ – H₂O₂ mixture was used.

The elemental concentration of vanadium is observed highest at 3.15, 3.54 and 5.0 when HNO₃ – H₂O₂, HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.6). The lowest concentration of vanadium is also observed at 0.33 (sample No. 1) only when HNO₃ - HClO₄ mixture was used and vanadium concentration could not be measured when HNO₃ – H₂O₂ , HNO₃ – H₂SO₄ mixtures were used.

The elemental concentration of molybdenum is observed highest at 0.61, 0.62 and 0.93 when HNO₃ – H₂O₂ , HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures are used respectively for the same sample (No.6). The lowest concentration of molybdenum is also observed lowest at 0.16 and 0.31 (sample No. 8) when HNO₃ – H₂SO₄ and HNO₃ - HClO₄ mixtures were used respectively and concentration of molybdenum could not be measured when HNO₃ – H₂O₂ mixture was used.

From the Tables 2, 3 and 4 it is worth mentioning that most of the elements for different samples could not be analyzed when HNO₃ – H₂O₂ to HNO₃ – H₂SO₄ mixture procedures were used. It indicates that the detection limit varies very greatly from HNO₃ – H₂O₂ to HNO₃ – H₂SO₄ to HNO₃ - HClO₄. It can be confirmed that HNO₃- HClO₄ mixture is highly suitable for detecting minute level of elemental concentrations in serum samples using ICP-AES.

Iron, Mn and Zn showed good recoveries for all 3-digestion procedures. Good recoveries were observed

for Cu and Pb in HNO₃-HClO₄ mixture. Low recoveries of Co, Ni, Mn, Cr, Cd, V and Mo were obtained in the two digestion procedures of HNO₃ – H₂O₂ to HNO₃ – H₂SO₄.

CONCLUSION

The Present study can be concluded with worth mentioning that most of the elements for different samples could not be analyzed when HNO₃ – H₂O₂ to HNO₃ – H₂SO₄ mixture procedures were used. It indicates that the detection limit varies very greatly from HNO₃ – H₂O₂ to HNO₃ – H₂SO₄ to HNO₃ - HClO₄. It can be confirmed that HNO₃ - HClO₄ mixture is highly suitable for detecting minute level of elemental concentrations in serum samples using ICP-AES. The concentration of Fe, Co, Ni, Mn, Cr, Cu, Cd, Pb, Mo and V in the serum of cataract patients was also determined by using ICP – AES technique in an effort to evaluate the status of these elements in such patients and to clarify their role in cataract disease. The main advantage of this procedure is that one can avoid the highly corrosive Hydrogen fluoride (HF) and it is very difficult to handle HF.

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