Fresenius J Anal Chem (2000) 368 : 627–632

ORIGINAL PAPER

C. Prohaska · K. Pomazal · I. Steffan

ETAAS method for the determination of Cd, Cr, Cu, Mn and Se in blood fractions and whole blood

Received: 20 March 2000 / Revised: 19 May 2000 / Accepted: 8 June 2000

Abstract An electrothermal atomic absorption method (ETAAS) for the direct determination of trace elements (Cd, Cr, Cu, Mn, Se) both in blood fractions (erythrocytes, plasma and lymphocytes) and whole blood was developed. Zeeman background correction and graphite tubes with L'vov platforms were used. Samples were diluted with HNO_3/Triton X-100 and pipetted directly into the graphite tube. Ashing, pretreatment and atomization steps were optimized carefully for the different fractions and elements applying different matrix modifiers for each element. For the lymphocyte fraction a multi-fold injection technique was applied. Low detection limits of the ETAAS method (Cd 0.13 μg/L, Cr 0.11 μg/L, Cu 0.52 μg/L, Mn 0.13 μg/L, Se 0.7 μg/L of whole blood) combined with small quantities of sample necessary for analysis allow determination of trace elements in this matrix. Verification of possible differences in the trace element status of humans was performed with statistical significance ($P < 0.05$). In addition, a contribution to the determination of normal values of essential elements was achieved. The method was applied for determination of trace elements in human blood and blood fractions of two groups ($n = 50$) different in health status.

Introduction

The trace elements Cr, Cu, Mn, and Se are known to be essential for humans. Therefore, the role of these elements in physiological processes is of interest.

The glucose tolerance factor (GTF) contains chromium. It is involved in the metabolism of carbohydrates probably by facilitating the binding of insulin to its receptor. Cr(III) is known to be biologically active and is mainly bound in blood to transferrin [1, 2].

C. Prohaska · K. Pomazal · I. Steffan (✉)
Institute of Analytical Chemistry, University of Vienna,
Waehringerstr. 38, 1090 Vienna, Austria

Manganese is involved in the metabolism of steroids and lipids as cofactor of the enzymes mevalonate kinase and farnesylpyrophosphate synthetase, two enzymes necessary for the biosynthesis of cholesterol. The most important enzyme containing manganese is Mn- superoxide dismutase, catalyzing the disproportioning of superoxide radicals. Manganese is bound in plasma to globulins [3].

Many determinations of selenium in body fluids are described in literature. The human metabolism of selenium, its concentration in plasma, urine and feces have been investigated. Concerning selenium in blood fractions, most of this element was found in plasma and erythrocytes [4, 5].

The toxic element cadmium is usually bound in blood to metallothioneins [6].

The concentrations of Cd, Cr, Cu, Mn, and Se in blood are well investigated [7–9], although few detailed studies about the concentrations in different blood fractions and especially about the binding of trace elements to certain proteins have been performed until now.

For the determination of the elements a suitable method has to be chosen. Using ETAAS (electrothermal atomic absorption spectrometry) it is possible to determine trace elements in very low concentrations in sample amounts of 20 to 100 μL. In literature whole blood and plasma are well investigated for almost all elements of interest, but very little is known about the cell fractions and the distribution of the elements in different cells. [7, 8] This is of great importance, because differences in the trace element status in whole blood and in blood fractions and in their distribution should be investigated in detail. The various correlations between the trace elements in the different blood fractions can bring new aspects for this topic with respect to different storage mechanism, kinetics, function, etc. [1–6].

Since problems are known causing difficulties for the determination of trace elements in biological matrices by ETAAS [7, 8], different chemical modifiers are used for the stabilization of the analyte. Using a L'vov platform furnace, atomizing is possible under nearly isothermal conditions. Ashing of the sample and Zeeman effect back-

ground correction are essential for a precise direct determination of trace elements in whole blood and blood fractions. Pyrolytically coated graphite tubes are favorable for chromium determination [10] and were used in this study as well.

The ETAAS method was optimized for the determination of Cd, Cr, Cu, Mn, and Se in whole blood and in blood fractions (plasma, erythrocytes and lymphocytes) of 7 control subjects. In the course of a clinical study Cr, Mn and Se were determined in whole blood and blood fractions of two groups of human subjects differing in health. Aim of this clinical study was to investigate the trace element status and to find differences in blood fractions of the two groups investigated.

Experimental

Chemicals and apparatus. All chemicals used were of reagent grade. Ficoll-Paque® from Pharmacia Biotech, Uppsala, Sweden; Heparin Immuno® 5.000 I.E./mL from Immuno, Vienna, Austria; nitric acid suprapur, pro Analysi (p.A.), Triton X-100, NaCl p.A and single element standards Titrisol® from Merck, Darmstadt, Germany. All solutions were prepared using doubly distilled water.
The apparatus used for measurements is described in Table 1.

Sample preparation. Blood samples were collected by Venflon™ (ported I.V. catheter, Becton Dickinson, Infusion Therapy Systems, Sandy, Utah, U.S.A.). The first 3 mL were discarded. Blood was filled in vessels previously tested for contamination and a defined amount of the anticoagulating agent heparin was added. It was checked for its content of elements of interest as well. For the separation of the blood fractions the reagents needed were prepared using doubly distilled water. Eppendorf vessels taken for storage of the blood fractions were tested for contamination, too. Erythrocytes were separated from the blood plasma by centrifugation (10 min at 1200 g) and washed three times using a physiological sodium chloride solution. Within 3 h after the blood drawing lymphocytes were separated by gradient centrifugation (30 min at 1200 g, Sorvall RT 6000B Refrigerated Centrifuge, Inula Vienna, Austria) from diluted blood (1:1 with physiological sodium chloride solution) with Ficoll-Paque®. Lymphocytes appeared as a ring approximately at half height of the solution in the tube and were collected carefully. They were washed three times with physiological sodium chloride solution and frozen in 1 mL aliquots at –20 °C for further use. The number of erythrocytes and lymphocytes per milliliter in the suspensions obtained were determined by a Coulter counter (Coulter Electronics limited, GB) to enable comparison of the results. The cell fractions were controlled optically by a microscope to ensure absence of other cells. Prior to analysis whole blood, erythrocytes, plasma and lymphocytes were thawed and homogenized in a Vortex mixer.

Table 1 ETAAS instrument parameters

Instrument:	GF-AAS: PE 4100 ZL
Spectrometer:	Littrow-design
Gratings:	1800 lines/mm
Atomizing unit:	Transverse heated graphite tube
Background correction:	Longitudinal Zeeman effect
Magnetic field:	0.9 Tesla
Inert gas:	Argon
Alternative gas:	Air
Autosampler:	AS-71
Sample volume:	20 µL

Table 2 Dilution ratios (sample : diluent, [v/v]) for ETAAS measurement

Fraction	Sample	HNO_3 (1% w/v)	Triton-X 100 (1% w/v)	H_2O
Whole blood	2	1	1	6
Plasma	2	1	1	6
Erythrocytes	8	1	1	–
Lymphocytes	8	1	1	–

Optimization of the temperature program. For the ETAAS determination suspensions of all samples in 0.1% nitric acid and 0.1% Triton X-100 were used (see Table 2). Temperature curves for Cd, Cr, Cu, Mn, and Se were registered in order to select the optimal pretreatment and atomization conditions for these elements in the four different matrices (whole blood, plasma, erythrocytes, and lymphocytes) compared to aqueous standard solution. For optimization the absorption signal was registered varying the temperature of the pretreatment and the atomization step. Matrix modifiers added were 50 µg $(NH_4)_2HPO_4$ + 3 µg $Mg(NO_3)_2$ for Cd, 15 µg $Mg(NO_3)_2$ for Cr and 5 µg Pd + 3 µg $Mg(NO_3)_2$ for Mn and Se.

Measuring conditions. Dilutions of whole blood, erythrocytes and blood plasma samples were injected directly into the graphite tube prior to measurement. Only for determinations in lymphocytes a volume of 20 µL was injected five times into the graphite tube performing drying steps (step 1 + 2) after each injection. Standard addition method was performed using the autosampler of the ETAAS apparatus and the optimized temperature program for whole blood, plasma, erythrocyte and lymphocyte samples, pipetting standards of different concentrations into the graphite tube, additionally to 20 µL of sample. The conditions for the measurement of Cd, Cr, Cu, Mn, and Se by ETAAS are listed in Table 3.

For all methods the detection limit was determined by measuring an appropriate reagent blank solution eleven times and a standard solution three times. The detection limit was calculated as the concentration equivalent to three times the standard deviation of the signal of the blank solution. All standard deviations are based on measurements in triplicate. Results are given as mean values +/– standard deviation. For validation of the optimized method whole blood reference material (IAEA-A-13) was analyzed.

Application. As application the concentrations of Cr, Mn, and Se were determined in blood and blood fractions (erythrocytes, plasma, lymphocytes) in two groups of human subjects (n = 50 each) differing in health. Using the computer program SPSS 7.5, statistical evaluation was performed. Significance was stated for $P < 0.05$ using a paired t-test following the requirements for the medical interpretation.

Table 3 General conditions for the ETAAS determinations

Element	Cd	Cr	Cu	Mn	Se
Wavelength [nm]	228.8	357.9	324.8	279.5	196.0
Lamp type	EDL[b]	HCL[a]	HCL[a]	HCL[a]	EDL[b]
Slit width [nm]	0.7	0.7	0.7	0.2	2.0
Modifier	$(NH_4)_2HPO_4$/Mg $(NO_3)_2$	–	–	–	Pd/Mg $(NO_3)_2$

[a] HCL: hollow cathode lamp; [b] EDL: electrodeless discharge lamp; "–" no modifier; 50 µg $(NH_4)_2HPO_4$, 3 µg $Mg(NO_3)_2$ and 5 µg Pd were used as matrix modifier

Table 4 Comparison of standard addition and direct measurement

Element	Mode	Whole blood [ng/mL]	Plasma [ng/mL]	Erythrocytes [ng/mL]	Lymphocytes [ng/mL]
Cr	Direct measurement	1.5 ± 0.3	0.8 ± 0.2	2.0 ± 0.3	0.2 ± 0.1
Cr	Standard addition	1.6 ± 0.2	0.7 ± 0.2	2.2 ± 0.4	–
Cu	Direct measurement	1340 ± 68	2083 ± 97	897 ± 36	27 ± 3
Cu	Standard addition	1405 ± 55	2113 ± 60	885 ± 45	–
Mn	Direct measurement	6.1 ± 1.4	1.1 ± 0.3	12.9 ± 1.4	0.7 ± 0.4
Mn	Standard addition	6.0 ± 1.2	0.9 ± 0.2	13.0 ± 1.8	0.5 ± 0.3
Se	Direct measurement	69.0 ± 3.4	75.2 ± 3.8	80.7 ± 4.4	0.9 ± 0.4
Se	Standard addition	72.0 ± 3.2	79.6 ± 2.7	83.5 ± 4.5	0.9 ± 0.3

"–" no data

Results and discussion

Sample preparation. The needle of the Venflon™ syringe is made of stainless steel and could cause contamination by the needle material. Therefore, it is removed immediately after the catheter is inserted. The number of cells per mL for erythrocyte and lymphocyte suspensions were used for quantification to be able to compare trace element concentrations in blood fractions. The results for erythrocytes and lymphocytes were calculated for 10^{10} and 9×10^6 cells per mL, respectively, since these are the mean concentrations found after cell separation. Washing procedures were performed three times to separate the cells completely and to obtain purified fractions. Purity of > 99% for cell fractions was set as limit. Contamination was prevented using glass ware purified by acid and blank solutions for correction of the analytical results.

The anticoagulating agent heparin was checked for the presence of the elements of interest. For lymphocytes and erythrocytes no additional contamination should be expected, because these cells are separated from the plasma and washed before analysis. The additional contamination caused by heparin was < 0.5% of the measured value for all elements in plasma and whole blood. Since concentrations of all elements investigated are low and relative standard deviations are about 5%, the contribution of heparin was not significant.

Optimization of the temperature program. Oxidation temperatures of less than 400 °C were not tested due to fast deposition of carbon in the graphite tube by blood residues at these temperatures. Increasing the ashing temperature from 400 °C to 600 °C no significant loss of the analytes could be observed. For ashing temperatures higher than 600 °C rapid oxidation of the graphite tube was observed. Since the decrease of the absorption signal was less than 3% compared to lower ashing temperatures and a lower background signal was produced during atomization using an ashing temperature of 600 °C, this temperature was chosen for measurement. Pretreatment temperatures were selected according to the highest possible value and the atomizing temperatures to the lowest possible value without lowering the analyte signal. Figures 1–4 are discussed in paragraphs dealing with the specific element. Graphite tubes were used for 300 firings in average. Possible losses of sensitivity caused by aging of the graphite tube during lifetime were corrected by measuring standard samples after every 10 blood samples.

Standard addition. Analyzing five samples from one person for each fraction the results obtained by standard addition were in good agreement with the results obtained by direct measurement (Table 4). Therefore, direct measurement was applied for the application study, since standard addition measurement needs larger sample amounts.

Cadmium. Even when adding matrix modifier (50 µg $(NH_4)_2HPO_4$ + 3 µg $Mg(NO_3)_2$) losses of the very volatile element Cd were observed for pretreatment temperatures higher than 700 °C (see Fig. 1). Therefore, 700 °C was chosen as pretreatment temperature in all matrices investigated. The highest sensitivity for Cd determination in blood matrix spiked with Cd standard was found for an atomization temperature of 1300 °C. In all matrices of interest low signal depression was observed in comparison to the aqueous standard. Concentrations were determined in blood of a group of young people (25 years in average) used for method development (n = 7, Table 5). RSDs are higher compared to other elements due to lower measuring concentrations. Most of the cadmium was found in erythrocytes (about 65%).

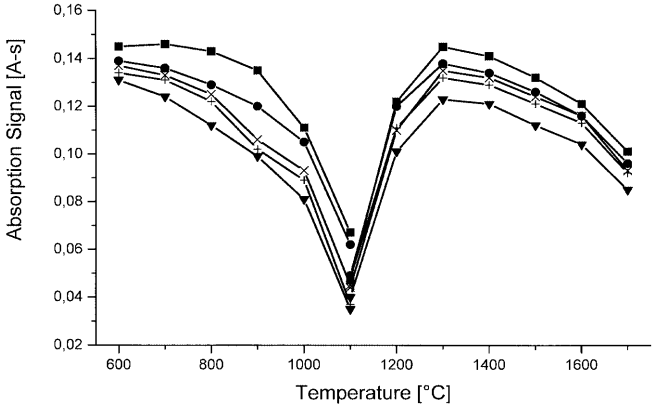

Fig. 1 Optimization curves for cadmium; ■ standard; + whole blood; **x** plasma; ▼ erythrocytes; ● lymphocytes

Table 5 Results of the method development study

Element	Whole blood ng/mL (RSD %)	Plasma ng/mL (RSD %)	% Whole blood (RSD %)	Erythrocytes ng/mL (RSD %)	% Whole blood (RSD %)	Lymphocytes ng/mL (RSD %)	% Whole blood (RSD %)
Cd	1.1 (59.4)	0.6 (85.6)	35.0 (26.7)	1.7 (64.0)	79.4 (44.3)	–	–
Cr	1.2 (18.2)	0.6 (32.9)	29.5 (12.3)	1.7 (12.5)	77.9 (16.3)	0.2 (36.9)	2.2 (1.1)
Cu[a]	1170 (41.8)	1630 (57.1)	72.7 (12.6)	870 (26.2)	36.5 (9.9)	–	–
Cu[b]	1170 (47.0)	1550 (60.7)	70.5 (13.2)	820 (34.4)	33.7 (7.5)	816 (59.3)	5.4 (1.7)
Mn	6.3 (25.4)	1.2 (55.1)	11.1 (8.0)	13.4 (21.3)	97.9 (12.3)	0.7 (64.5)	1.3 (1.1)
Se	80.4 (18.6)	70.5 (21.7)	48.1 (4.2)	95.0 (19.8)	56.4 (6.2)	5.7 (94.1)	0.5 (0.4)

"–" no data. [a] ETAAS, [b] ICP-OES

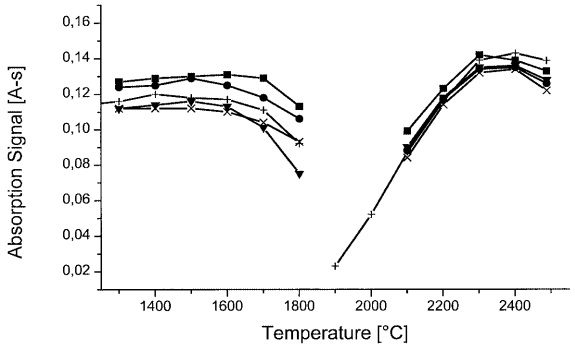

Fig. 2 Optimization curves for chromium; ■ standard; + whole blood; **x** plasma; ▼ erythrocytes; ● lymphocytes

Chromium. Atomization and pretreatment temperatures showing the highest absorption signals for chromium in matrix were chosen for the temperature program (see Fig. 2). During the pretreatment step a loss of analyte occurred for temperatures above 1500 °C in all fractions. Therefore, this temperature was chosen for measurement. Optimizing the atomization temperature the highest sensitivity was observed between 2300 and 2400 °C. Since higher atomization temperatures shorten the lifetime of the graphite tube 2300 °C was chosen for atomization. Adding 15 µg of $Mg(NO_3)_2$ per injection as matrix modifier did not improve sensitivity or peak shape compared to measurement without modifier. Therefore, no modifier was used for the final measurement. The optimized temperature program is shown in Table 6. The detection limit in whole blood matrix was 0.11 µg Cr/L. For lymphocytes a multi-fold injection technique (total sample volume 100 µL) was applied to improve the detectable concentration in the measuring solution [11]. For validation of concentrations measured in whole blood only IAEA-A-13 freeze dried animal blood was available. Results obtained applying the optimized method (14.1 ± 3.9 ng/g) showed no significant differences to published results (15.0 ± 4.5 ng/g) [12]. Results of the clinical study are shown in Table 7. Most of the chromium found is located in the erythrocytes. Interestingly, only small amounts of chromium are found in plasma, although the most important biological function of chromium is supposed to be as glucose tolerance factor in carbohydrate metabolism. In lymphocytes a relatively large amount of the total chromium (3.8%) was found, similar results are reported in literature [13]. Significant differences ($P < 0.05$) in the concentration of chromium in blood fractions of the two groups of the clinical study were found in plasma according to the medical

Table 6 Temperature program for Cd, Cr, Cu, Mn, and Se

Element	Step	Temp. [°C]	Ramp [s]	Hold [s]	Gas flow [mL/min]	Gas	Remark
	1	110	1	25	250	Ar	Drying
	2	130	5	25	250	Ar	Drying
	3	600	10	20	250	Air	Ashing
	4	600	10	10	250	Ar	Flushing
Cd	5	700	10	10	250	Ar	Pretreatment
	6	1300	0	5	0		Atomization
Cr	5	1500	10	10	250	Ar	Pretreatment
	6	2300	0	5	0		Atomization
Cu	5	1200	10	10	250	Ar	Pretreatment
	6	2100	0	5	0		Atomization
Mn	5	1300	10	10	250	Ar	Pretreatment
	6	1900	0	5	0		Atomization
Se	5	1100	10	10	250	Ar	Pretreatment
	6	1900	0	5	0		Atomization
	7	2400	1	2	250	Ar	Cleaning

Table 7 Results of the clinical study

Group 1								
Element	Whole blood [ng/mL]	SD	Plasma [ng/mL]	SD	Erythrocytes[a] [ng/mL]	SD	Lymphocytes[b] [ng/mL]	SD
Cr	1.29	0.86	0.75	0.55	2.21	0.60	0.60	0.06
Mn	7.89	3.19	1.81	1.38	13.26	7.21	0.51	0.27
Se	71.83	18.11	72.31	17.56	83.11	33.50	0.85	0.51
Group 2								
Element	Whole blood [ng/mL]	SD	Plasma [ng/mL]	SD	Erythrocytes[a] [ng/mL]	SD	Lymphocytes[b] [ng/mL]	SD
Cr	1.23	0.81	1.10	0.81	2.36	1.58	0.64	0.06
Mn	8.40	3.38	1.56	0.98	16.05	6.72	0.67	0.28
Se	68.19	19.26	66.25	18.64	86.30	29.61	1.14	4.81

[a] calculated for 10^{10} cells per mL for comparison of results
[b] calculated for 9×10^6 cells per mL for comparison of results

Table 8 Comparison with concentrations given in literature

Element	Whole blood		Serum	
	Measured [ng/mL]	Lit. [2–9] [ng/mL]	Measured [ng/mL]	Lit. [1, 4–9] [ng/mL]
Cd	1.1	1–2	0.6	0.7
Cr	1.2	1–10	0.6	0.2–0.8
Cu	1170	800–2000	1590	200–2000
Mn	6.3	6–12	1.2	0.3–3
Se	80.4	70–240	70.5	20–150

requirements. The medical interpretation of this results will be published elsewhere. Concentrations given in literature vary over a wide range, probably due to higher concentrations measured in the past using methods not sufficiently sensitive and to contamination caused by stainless steel needles used for blood drawing (Table 8).

Copper. Optimization of the pretreatment and atomization temperature for copper revealed that the matrix did not change the behavior of the analyte significantly during pretreatment (Fig. 3), but for atomization a higher temperature than recommended by the producer [14] was found to be optimal in all fractions (Table 6). For comparison measurements by ICP-OES (DL = 2.5 µg Cu/L) were performed and were in good agreement with the concentrations determined by ETAAS (DL = 0.52 µg Cu/L), (see Table 5). For the clinical study ICP-OES was chosen for measurement because of the higher sample throughput using this method [15].

Manganese. Atomization and pretreatment temperature curves had a similar shape for the aqueous standard and all blood fractions (Fig. 4). Temperatures showing the highest absorption signals for manganese in matrix were chosen for the temperature program (1300 °C for pretreatment and 1900 °C for atomization, Table 6). Pd in combination with $Mg(NO_3)_2$ and added individually, as found in literature [16, 17], was tested as modifier, but did improve neither sensitivity nor peak shape. The detection limit in whole blood matrix was 0.13 µg Mn/L, results obtained applying the optimized method (42 ± 4 ng/g for analyzing the reference material IAEA-A-13) were not significantly different compared to published results (39 ±

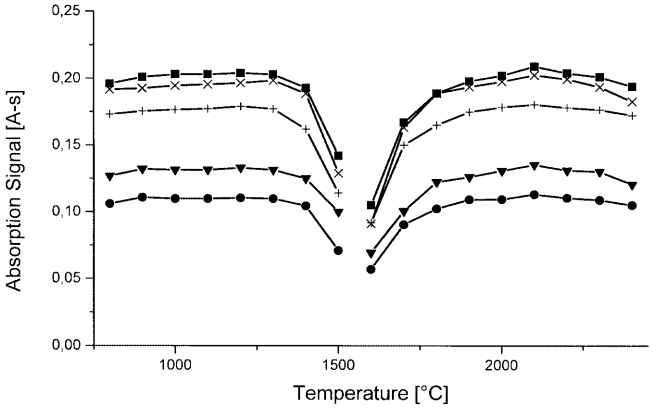

Fig. 3 Optimization curves for copper; ■ standard; + whole blood; **x** plasma; ▼ erythrocytes; ● lymphocytes

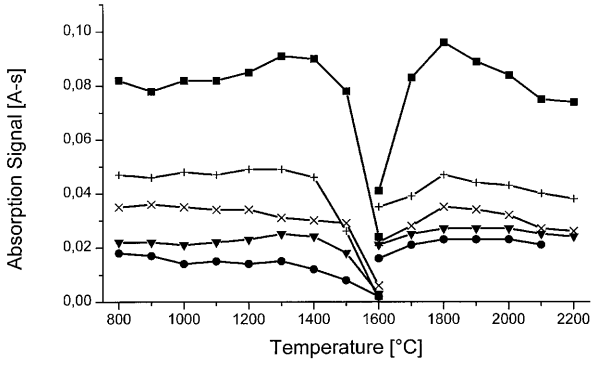

Fig. 4 Optimization curves for manganese; ■ standard; + whole blood; **x** plasma; ▼ erythrocytes; ● lymphocytes

5 ng/g) [12]. Most of the manganese in blood is located in erythrocytes (about 90%). Therefore, determination in whole blood or erythrocytes seems reasonable, even more because of the very low concentrations of this element found in plasma. Results of the clinical study are shown in Table 7. Significant differences ($P < 0.05$) in the concentration of manganese in blood fractions of the two groups of the clinical study were found for lymphocytes according to the medical requirements. The medical interpretation of this results will be published elsewhere.

Selenium. The detection limit in whole blood matrix was 0.7 μg Se/L. The temperature program is shown in Table 6. Optimization of the method was published elsewhere [18].

Conclusion

An analytical procedure for the determination of trace elements in whole blood and blood fractions was established in order to obtain information about the status of trace elements in humans. AAS was adapted for the measurement of Cd, Cr, Cu, Mn, and Se in these matrices. In the course of a clinical study about essential elements ETAAS was applied for the determination of Cr, Mn, and Se in whole blood, plasma, erythrocytes and lymphocytes of approximately 100 human subjects. The relatively high amounts of copper present in blood were determined by ICP-OES. For each element the temperature program and addition of modifiers were optimized. ETAAS enables determination of element concentrations < 1 ng/mL. The method enables the determination of Cr, Mn and Se in the very low concentrations present in blood. The concentrations measured are within the range of data published in literature (Table 8). The concentrations measured in whole blood could be verified by the measurements in the blood fractions (sum: 104.2 ± 11.8% of the total amount in whole blood). The medical and biochemical aspects of the application study will be published elsewhere.

Acknowledgments The authors wish to thank Prof. W. Marktl and Prof. G. Schernthaner for their kind support, and the "Medizinisch-wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien" for financial support.

References

1. Ducros V (1992) BioTrace Elem Res 32: 65
2. Morris BW, Griffiths H (1990) Clin Chem Enzym Comms 2: 61
3. Johnson JM, Kies C, Fox HM (1988) Nutr Rep Int 37: 1297
4. Patterson BH, Levander OA, Helzlsouer K, McAdam PA, Lewis SA, Taylor PA, Veillon C, Zech LA (1989) Am J Physiol 257: R556
5. Alaejos MS, Romero CD (1995) Chem Rev 95: 227
6. Bertram HP (1992) Spurenelemente, Urban & Schwarzenberg, München Wien Baltimore
7. Taylor A, Branch S, Halls DJ, Owen LMW, White M (1998) J Anal At Spectrom 13: 57R–106R
8. Taylor A, Branch S, Crews HM, Halls DJ, Owen LMW, White M (1997) J Anal At Spectrom 12: 119R–221R
9. Hill SJ, Dawson JB, Price WJ, Shuttler IL, Smith CMM, Tyson JF (1998) J Anal At Spectrom 13:131R–170R
10. Rubio R, Sahuquillo A, Rauret G, Quevauviller P (1992) Intern J Environ Chem 47: 99–128
11. Bermejo-Barrera P, Moreda-Pineiro J, Moreda-Pineiro A, Bermejo-Barrera A (1998) Fresenius J Anal Chem 360: 208–212
12. Rodushkin I, Ödman F, Branth S (1999) Fresenius J Anal Chem 364: 338–346
13. Schelenz R, Parr RM, Zeiller E, Clements S (1989) Fresenius Z Anal Chem 333: 33–34
14. Perkin Elmer (1992) Techniques and recommended conditions for THGA graphite furnace
15. Prohaska C, Pomazal K, Steffan I (2000) Fresenius J Anal Chem 367 : 479–484
16. Luna AS, Calixto de Campos R (1999) At Spectrosc 20:108–112
17. Moreira FR, Pivetta F (1998) At Spectrosc 19: 137–142
18. Prohaska C, Steffan I, Pomazal K, Törvényi A (2000) J Anal At Spectrom 15: 97