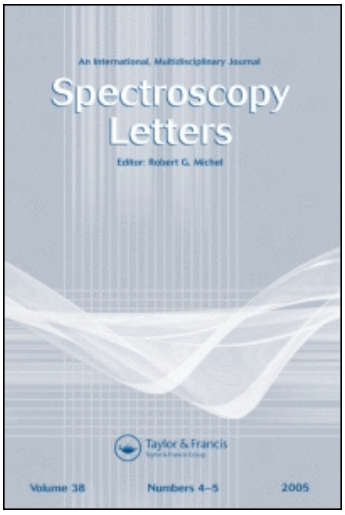

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Analytical Scheme for the Direct Graphite-Atomizer/Flame Atomic Absorption Spectrometric Determination of Fifteen Trace Elements in Toenails for Biological Monitoring

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ANALYTICAL SCHEME FOR THE DIRECT GRAPHITE-ATOMIZER/FLAME ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF FIFTEEN TRACE ELEMENTS IN TOENAILS FOR BIOLOGICAL MONITORING

KEY WORDS: Atomic Absorption Spectrometry, Graphite Atomizer, Flame AAS, Trace Elements, Nails, Hair, Solubilization, Tetraalkylammonium Hydroxide, Chemical Modification, Biological Monitoring.

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ABSTRACT

An analytical scheme for the determination of up to fifteen trace elements in nails and hair has been proposed. Samples were solubilized by means of aqueous tetraalkylammonium hydroxide (alkyl = methyl or ethyl). Cadmium, Cr, Cu, Fe, Mn, Pb and Zn were determined by pulse-nebulization flame AAS, employing the Slotted Tube

Atom Trap (STAT) for Cd and Pb. Suitable chemical modifiers have been applied in electrothermal AAS (ETAAS) determinations of Cd and Pb (ammonium hydrogen phosphate), As, Sb, Se and Sn (palladium), while no modifier was needed in determinations of Al, Co, Cr, Mn, Mo and Ni. Calibration was performed by means of matrix-matched standards and the technique was verified by standard additions, comparison with neutron activation and analysis of hair reference material. The usefulness of this technique for occupational exposure monitoring is briefly discussed.

INTRODUCTION

The environmental/occupational impact on human health has been a serious concern during the last decades and biological monitoring by means of analytical AAS determination of trace elements in human biological samples has significantly improved[1,2]. It has been realized that elemental analysis of certain skin keratin appendages (hair, nails) as human biopsy material may offer distinct methodological and analytical advantages:

1. Non-invasive, easy and painless sampling, without any biological hazard to the donor.

2. Convenience in transportation and storage of samples.

3. Relatively high concentrations of most elements (vs. e.g. blood, serum or urine), resulting in more

favorable analyte-to-matrix ratios, less pronounced interferences as well as lower contributions of blank and sporadic contamination during analysis.

4. Samples are relatively easily brought into solution.

On the other hand, the usefulness and reliability of toxicokinetic tests based on analysis of hair and nails could be limited, due to at least two intrinsic problems with these biopsy materials: large biological variability and substantial exogenous contamination of hair and nails. Currently, there seems to be no reliable washing procedure for hair so as to remove only the exogenous contamination without extracting the endogenous trace elements. The problem is particularly serious in the field of occupational monitoring. Therefore, toenails were expected to be more suitable biological material for monitoring purposes because of the better concistence of nail samples and the possibility to apply a combined mechanical and chemical cleaning procedure.

The review of the existing literature[1,2] indicated that up to twenty elements should be most important to determine from the viewpoint of occupational and environmental monitoring as well as medical, pharmacokinetic and forensic interest: Al, As, Au, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V and Zn.

The aim of this study was to develop an analytical scheme for the determination of a number of relevant trace elements in toenails with a view to biological monitoring. The current technique-of-choice in this field, AAS, has been applied, taking into consedetration the limited amount of available sample (ca. 0.10-0.15 g per month) and therefore employing miniaturized solubilization procedure with micro techniques of AAS (pulse-nebulization and graphite-atomizer AAS).

EXPERIMENTAL

Apparatus

Several atomic absorption spectrophotometers were used throughout this study: a Pye Unicam SP 1950 double beam instrument, a Pye Unicam SP 9-800 with SP 9 computer (both in flame mode and with a STAT accessory; STAT = Slotted Tube Atom Trap), a Perkin-Elmer model 2380 G spectrometrer with a HGA-400 Heated Graphite Atomizer, and a model P-E 1100 B with HGA-700. Background correction (deuterium lamp) was required except for the determinations of Zn by flame AAS and Cr, Mn and Ni by ETAAS. The operating parameters were set as recommended by the manufacturer except that a bandpass of 2 nm and alternative wavelengths were used for Sb (231.2 nm) and Sn (286.3 nm). Uncoated graphite tubes were used except for molybdenum (pyrocoated tube).

TABLE 1

Conditions for the Determination of Groups of Analytes in TAAH-solubilized Samples

Analyte	Matrix % m/V	Technique	Comments
Zn	1	FAAS	Pulse-nebulization (PN)
Cr,Cu,Fe,Mn	10	FAAS	PN, 100 μl
Cd,Pb	10	FAAS	PN and STAT
Al,Cr,Mn,Ni	1	ETAAS	
Cd,Pb	1	ETAAS	+ 50 μg $(NH_4)_2HPO_4$
Co,Mo	10	ETAAS	
As,Sb,Se,Sn	10	ETAAS	+ 20 μg Pd(II)

For pulse-nebulization AAS, a PTFE microsampling cup was attached to the nebulizer by means of a short PTFE capillary (ca. 5 cm); 100 to 150-μl sample aliquots were manually injected by means of a micropipette (Table 1). The optimized temperature programs for the HGA are shown in Tables 2 and 3.

Reagents

40 % tetraethylammonium hydroxide in water (aq.TEAH, pract. grade, Fluka), 25 % tetramethylammonium hydroxide in water (aq.TMAH, pract., Fluka), 25 % tetramethylammonium hydroxide in methanol (TMAH-MeOH, pract., Fluka), 10 % TMAH in water (aq. TMAH, p.a., Merck) and

TABLE 2

Temperature Program for the HGA-400

Heated Graphite Atomizer

(Al, Cd, Cr, Mn, Ni and Pb in 1 % m/V homogenates)

Step	Temp. °C	Time[a] s	Ar flow ml/min	Read
1	130	25 + 20	300	off
2	var[b]	15 + 10	300	off
3	var[c]	10 + 40	300	off
4	var[d]	0 + 3	50	on
5	2600	1 + 2	300	off

[a] ramp + hold time;
[b] variable: Cd 400°, Al, Cr, Mn, Ni and Pb 550°;
[c] variable: Cd 600°, Pb 750°, Mn and Ni 1050°, Al and Cr 1100°;
[d] variable: Cd 1600°, Pb 1800°, Al, Cr, Mn and Ni 2400°.

Lumatom (Solubilizer for microelement analysis containing quaternary ammonium hydroxide in toluene, H. Kürner, Rosenheim, Germany), Triton X-100 (Fluka), diammonium hydrogen phosphate (p.a., Merck), palladium (II) as metal dissolved in HNO_3 + HCl, and doubly distilled water.

Analytical Procedure

Nail clippings were thoroughly scraped by means of a quartz wafer so as to remove a thin surface layer,

TABLE 3

Temperature Program for the HGA-400

Heated Graphite Atomizer

(As, Co, Mn, Sb, Se and Sn in 10 % m/V homogenates)

Step	Temp. °C	Time[a] s	Ar flow ml/min	Read
1	100	20 + 5	300	off
2	130	10 + 10	300	off
3	300	25 + 5	300	off
4	600	10 + 10	300	off
5	var[b]	5 + 30	300	off
6	var[c]	0 + 3	0	on
7	2600	1 + 2	300	off

[a] ramp + hold time;
[b] variable: Co and Se 1100°, As, Sb and Sn 1200°, Mo 1800°;
[c] variable: As, Sb and Se 2400°, Co and Sn 2500°, Mo 2650°.

considered as endogenously-contaminated. The weighed sample (ca. 0.05-0.1 g) was placed in a tall, graduated quartz tube and was washed for 1 min under intensive vortex mixing with two successive portions of 3 ml of aq. 0.1 % m/V Triton X-100, followed by three rinses with doubly distilled water, each for 1 min.

Samples were solubilized with 0.5 ml of aq. 40 % TEAH (ca. 100 µl of base per 20 mg of sample) at 90°C

(water bath) for approximately 30 min. Solubilized samples were diluted with doubly-distilled water to 5 or 10 ml , as appropriate, so as to obtain 10 % m/V sample homogenates. Aliquots of homogenates were directly injected into the graphite atomizer (10-30 μl) or into the microsampling cup for the pulse-nebulization flame AAS (100-150 μl), or otherwise were further diluted or mixed with an appropriate chemical modifier (Table 1).

RESULTS AND DISCUSSION

The recommended procedure for cleaning/washing of toenail samples was developed on the basis of preliminary experience with manganese determinations[3] . Manganese was deemed among the best examples of an ubiquitous element, exhibiting a gross exogenous contamination of samples (e.g., the ratio of Mn concentrations in the Earth's crust ($\approx$ 1000 μg/g) to the nail ($\approx$ 1 μg/g) is rather high - ca. 10^3). Therefore, the plain washing could not rule out the systematic errors due to exogenous contamination of samples. A combined cleaning/washing procedure has been adopted, involving a mechanical scraping of the surface layer of the nail clipping as well as successive washings with an aqueous non-ionic detergent and water. The mass loss during this washing was about 3.5-4.5 % and the sample preparation rate was about 7-8 h^{-1}.

Several solubilization reagents have been tested, consedering the speed of sample homogenization, the blank levels, the effect of base/solvent on sensitivity and the convenience of handling of reagents and sample homogenates.

Generally, samples of nails (N) and hair (H) were solubilized faster (within 30 min) with aqueous solutions of quarternary ammonium hydroxides and much slower (up to several hours) with TAAH in organic solvents:

N-aq.40% TEAH < N-aq.25% TMAH < H-aq.40% TEAH <
< H-aq.25% TMAH < N-25% TMAH-MeOH < H-25% TMAH-MeOH <
< H-Lumatom < N-Lumatom.

Solubilization could be facilitated and sped up, as well as could be performed at lower temperatures (70^{o}C) by an overnight soaking of samples in the base reagent and/or by applying occasional, brief vortex mixing during solubilization.

Reagent blanks were generally lower with organic solvents, e.g.: Lumatom < TMAH-MeOH < aq. TMAH. Because of the leaching of trace impurities from the reaction vessels by alkaline reagents, it has proved essential to use test tubes made of quartz rather than of glass (e.g. quartz vessels provided 3-fold (Mn, Pb) to 30-fold (Al) lower blank levels). The typical blanks for final sample solutions in aq. TAAH were (in ng/ml):

Al 25, As 1.1, Cd 0.2, Cr 3, Mn 3, Ni 1.4, Pb 4.6, Mo 1, Sb 0.9, Se 2.1, and Sn 3.4 .

Batches of 10 to 30 base digestions were conveniently run on a thermostated water bath. The need for efficient aspiration of the evolved toxic vapors of TAAH and organic solvents should be stressed on.

The sensitivity of flame AAS determination depended within a factor of two on the transport properties of the solubilized samples (viscosity, surface tension) and could be approximately ranked as follows:

TMAH-MeOH < aq. TEAH < aq. TMAH < Lumatom-EtOH ≈ aq.std.

Aqueous TAAH reagents proved to be the most convenient for routine practice and all further work was done with aq. TEAH or TMAH. The alkaline homogenates exhibited a limited time stability, presumably due to stratification and hydrolysis of analytes and were therefore analysed within the same working day.

Sample homogenates were thoroughly mixed before sampling. Calibration was performed by means of matrix-matched standards, obtained by spiking a hair or nail pool with known additions of the analytes.

The instrumental sample throughput was 22 h^{-1} and 26 h^{-1}, with 10 % (m/V) and 1 % (m/V) sample homogenates, respectively (Tables 2 and 3).

The procedure has been validated by means of standard additions and recovery tests. Portions of

TABLE 4

Analysis of Reference Materials and Figures of Merit (in $\mu g/g$)

Analyte	Method	Reference Data	Found[a]	RSD[b] %	Error %	LOD	ULR[c]
Al	ETA	240	252 ± 21	5.2	5.0	3	40
As	ETA	0.0375[d,e]	0.0318[e]	-	-15	0.03	0.5
Cd	ETA	0.2 ± 0.03	0.201 ± 0.031	11	0.5	0.03	0.3;1[f]
Cr	ETA	1.4 ± 0.2	1.48 ± 0.21	8.6	5.7	0.05	2
Cu	F	16.3 ± 1.2	16.3 ± 0.6	2.4	0	0.1	-
Mn	ETA	5.2 ± 0.3	4.99 ± 0.14	1.7	-4.0	0.2	3.5;6[f]
Mn	F	5.2 ± 0.3	5.36 ± 0.33	4.0	6.9	0.13	-
Ni	ETA	1.8 ± 0.1	1.87 ± 0.17	6.1	3.9	0.04	1.5
Pb	ETA	6.0	5.6 ± 0.4	4.8	-6.7	0.3	2;20[f]
Sb	ETA	(0.07)[g]	0.0705[e]	-	0.7	0.02	1
Se	ETA	0.70;0.92[d,h]	0.80[e]	-	-1.9	0.02	1
Sn	ETA	1.62[h];1.58[i]	1.52[e]	-	-0.5	0.02	0.8

[a] mean ± confidence interval (P = 0.05);
[b] n = 4;
[c] ULR = upper linear range;
[d] RNAA result from an external laboratory;
[e] mean of two parallels;
[f] argon flow 50 ml/min ("mini-flow");
[g] information value only;
[h] from Reference 5;
[i] from Reference 6.

pooled, homogenized hair sample were analyzed by this procedure as well as by radiochemical neutron activation analysis (RNAA) (Dr. M. Dermelj, University of Ljubljana, Slovenia). A certified reference material, CRM No. 5 "Human Hair" from the National Institute for Environmental Studies (NIES), Tsukuba, Japan[4] has also been analyzed for a number of elements. The results are in a good agreement, as shown in Table 4, together with the precision and accuracy data, the limits of detection (3 σ-criterium) and the upper linearity range.

Unfortunately, no certified reference material on a nail base is available to further validate the procedure but the matrix of hair and nail is known to be rather similar as far as the sample preparation and AAS assay is considered[1,2].

This analytical scheme has been applied to biological monitoring in a large-scale study during the last several years. A total of 262 persons (207 non-exposed and 55 with known occupational exposure), splitted in 14 control groups and 6 exposed groups have been studied. Part of the results for the content of trace elements in toenails of persons without known occupational or environmental exposure are shown in Table 5. These results are in agreement with the literature data[2] and could be considered as a "control group" reference data.

TABLE 5

Results for the Trace Elements Concentration in Toenails of Control Groups of Occupationally Non-exposed Individuals (in μg/g)

Element	Mean ± SD	Median	Range	No. of controls and sex
Al	27.4 ± 32.8	15.1	1.1–122	24 (M)
As	0.58 ± 0.44	0.36	0.13–1.60	18 (M)
Cd	0.13 ± 0.17	0.08	0.01–0.91	29
	0.13 ± 0.20	0.08	0.01–0.91	19 (M)
	0.12 ± 0.12	0.08	0.02–0.39	10 (F)
Co	0.027 ± 0.012	0.026	0.013–0.053	21
	0.024 ± 0.010	0.021	0.013–0.051	15 (M)
Cr	1.3 ± 1.3	0.9	0.3–7.6	42
	1.0 ± 0.6	0.8	0.3–2.1	21 (M)
	1.6 ± 1.7	1.0	0.4–7.6	21 (F)
Cu	2.6 ± 1.4	2.4	0.1–6.0	18
	3.0 ± 1.4	2.4	1.8–5.7	7 (M)
	2.5 ± 1.5	2.4	0.1–6.0	11 (F)
Fe	13.4 ± 10.1	10.8	4.2–48.7	41
	12.6 ± 6.6	13.6	4.2–31.5	22 (M)
	14.2 ± 13.2	9.9	4.2–48.7	19 (F)
Mn	0.8 ± 0.5	0.6	0.1–2.7	49
	0.9 ± 0.5	0.8	0.2–2.7	26 (M)
	0.6 ± 0.4	0.5	0.1–1.7	23 (F)
Mo	0.061 ± 0.032	0.062	0.017–0.127	20

(continued)

TABLE 5 (cont'd)

Ni	0.23 ± 0.22	0.14	0.04-1.04	30
	0.20 ± 0.16	0.16	0.04-0.49	22 (M)
Pb	1.7 ± 1.9	1.0	0.1-8.8	40
	1.1 ± 0.9	0.9	0.2-2.3	19 (M)
	2.3 ± 2.3	1.6	0.1-8.8	21 (F)
Sb	0.35 ± 0.17	0.31	0.021-0.70	20 (M)
Se	0.34 ± 0.18	0.34	0.05-0.61	16
Zn	87.7 ± 21	86.0	50.3-149	24
	83.3 ± 20.2	83.1	50.3-129.6	15 (F)

Other relevant tests (Pb in blood, Cr, Mn and Pb in urine) as well as analyses of air have been performed but are outside the analytical scope of this paper. Their results and discussion have been reported or published elsewhere for Mn[3,7], Cr[8], Pb[8], Zn[9], etc.[9], indicating on the usefulness of these new toxicokinetic exposure test for biological monitoring (on a group basis).

CONCLUSION

The successive determination of fifteen trace elements in toenails (and hair) by pulse-nebulization flame AAS and graphite-furnace AAS after solubilization with tetraalkylamonium hydroxide is straightforward. A

time-limiting stage of this assay is the removal of the exogenous surface contamination of toenail samples by means of a combined scrapping/washing procedure. The technique provides accurate and precise data and is promising for biological monitoring of trace elements, e.g. chromium, manganese and lead.

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