

X-ray Diffraction and Elemental Analysis of Medical and Environmental Samples

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The results of the elemental and chemical composition analysis of human medical samples (blood, serum, hair, urine, tooth, kidney stones, gallstones) and environmental samples (slag, cereal, vegetables, flour, pork bones, pork meat, fish) are presented. The analysis were performed by application of the total reflection X-ray fluorescence, wavelength dispersive X-ray fluorescence and X-ray powder diffraction methods. With X-ray fluorescence methods the following elements were identified: O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, Zr, I, Ba, and Pb, whose concentrations were from a few ng/g to tens of percent. For some samples the elemental analysis was extended by X-ray powder diffraction measurements. With this method the chemical composition was determined. In the paper the experimental setups, methodology of samples preparation and methods of carrying out the measurements are described. As an example the X-ray spectra registered for gallstone sample are discussed in detail. Finally, the results of X-ray diffraction and elemental analysis for selected medical and environmental samples are summarized.

DOI: [10.12693/APhysPolA.125.911](https://doi.org/10.12693/APhysPolA.125.911)

PACS: 78.70.En, 87.64.Bx, 32.30.Rj, 87.64.kv

1. Introduction

Adequate content of the elements and compounds in the human body is of fundamental importance for its healthy functioning [1]. Both excess and deficiency of the elements and compounds can lead to their abnormal redistribution and accumulation, which in turn, can have a significant impact on human health. The level of elements and compounds in the human body is strongly influenced by their contents in the environment, pollution of the environment and individual nutrition [1–3]. Correlating of content of the trace elements and compounds in medical samples, such as, for example: bones, teeth, solid concretion, or crystal aggregation in human body, with the same content measured in environmental samples (food, air, water, soil, plants) allows to investigate the dynamics of elements transport, accumulation processes and, generally, the influence of environmental pollution on a level of trace elements and compounds in human organism [1–3].

The elemental and chemical analysis of medical and environmental samples can be performed by many different analytical methods [4, 5], especially by X-ray fluorescence and X-ray powder diffraction techniques [6–10]. The main aim of presented studies is analysis of the elemental and chemical composition of human medical samples (blood, serum, hair, urine, tooth, kidney stones, gallstones) and environmental samples (slag, cereal, vegetables (parsley, carrot, cabbage), flour, pork bones, pork meat, fish) by application of the total reflection X-ray

fluorescence (TXRF) [11], wavelength dispersive X-ray fluorescence (WDXRF) [12] and X-ray powder diffraction (XRPD) [13] methods. The measurements were performed in the Institute of Physics of Jan Kochanowski University (UJK) in Kielce (Poland). The performed complementary WDXRF and TXRF analysis allowed to mark the element concentrations in medical and environmental samples in wide range of elements (from oxygen to lead) and broad range of the concentrations (from tens of ppb to tens of percent). On the other hand, analysis with the XRPD method has provided qualitative information about the compounds composition of the analyzed samples.

In this work the systems setups, methodology of samples preparation and methods of carrying out the measurement will be described. The paper is summarized by interpretation of obtained results.

2. Methods of analysis

The WDXRF and TXRF methods are modifications of well known X-ray fluorescence analysis [14] which bases on excitation of characteristic X-rays in a sample as a result of interaction of primary X-ray beam with sample atoms. Detection and analysis of these characteristic X-rays give both quantitative and qualitative information about sample elemental composition.

The XRPD method allows for analysis of sample chemical composition by interpretation of the primary X-ray beam diffracted on the sample crystal structure.

2.1. WDXRF method

The elemental composition measurements with WDXRF technique were performed using Axios spectrometer (PANalytical) equipped with Rh anode X-ray tube with maximum power 2.4 kW. The wavelength

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dispersive system of the spectrometer used five crystals (LiF (200), Ge (111), PE (002), PX1, LiF (220)) which were automatically selected during the measurements. The characteristic X-rays induced in the sample were diffracted on one of the crystals and measured by flow proportional counter for optimal detection of elements up to Fe or a scintillation detector for heavier elements.

In order to cover the X-ray energy (wavelength) range of interest it was necessary to perform 12 scans with different diffraction crystal–detector configurations. Energy resolution of the setup (10–50 eV) allows for unambiguous identification of element intensity even for very rich elemental composition samples. The measurements were performed in vacuum (solid samples) or helium (powder samples). The quantitative analysis of the spectra was performed with the PANalytical analytical program Omnian [15]. The Omnian package is available for the standardless analysis of all types of samples. Omnian is a combination of advanced software and setup samples, whose an installation is achieved by the measurement of the setup standards. These well chosen materials are designed for even the most challenging of matrices. Made from pure starting materials the setup standards are used to fine-tune Omnian software to the spectrometer subtleties while incorporating spectral elements features that overcome the limitations of other semiquantitative strategies. Omnian software includes advanced 3rd generation FP model algorithms and it is the union of these two features which make the core difference. The result is a method which can be used for all sample types whether they are liquids, powders or solids providing robust and accurate elemental analysis [15].

Omnian includes advanced algorithms designed to profile known limitations inherent to XRF. For example Omnian results will only include elements which have been detected and determined to be significant. Also upon examination Omnian automatically employs a strategy to overcome spectral interference, whenever advantageous possible interference free lines will be chosen and corrections will be automatically applied. Omnian also includes a full suite of advanced matrix corrections, for example variable sample thickness compensation. Also, the dark matrix correction provides better accuracy in cases where light elements such as C, H, and O contribute to significant absorbance. Fluorescent volume geometry (FVG) is another advanced matrix correction for spectral contributions where heavy elements are measured in light matrices.

Corrections which can be involved in Omnian quantitative analysis are the following: (a) finite thickness (correction where the sample is not infinite thick for all measured energies), (b) normalization (results without normalization, normalization to required sum, calculation of one compound by difference), (c) the Compton validation factor (analysis of unmeasured matrix compounds by using the Compton-scattered tube intensity; Omnian can use this factor to calculate the concentration of unmeasured matrix compounds), (d) FVG correction (determi-

nation of the geometry of the optical path), (e) medium correction (the medium in which the WDXRF measurements were performed), (f) compound list (enables to select a compound list when compounds such as oxides, sulfides etc. instead of elements are analyzed), (g) sample preparation (enables to define the sample preparation parameters such as binders, fluxes and sample weights).

Application of the WDXRF allowed for elemental analysis from oxygen (O) to lead (Pb) in wide range of concentrations, with detection limit on the level of about 10 $\mu\text{g/g}$. In the context of performed studies the main advantages of the WDXRF method are possibility of the concentration determination for light elements (O, Na, Mg, Al, Si) and good resolution of the characteristic X-ray lines. Taking into account the form of analyzed samples the following solid samples were analyzed: slag, cereal, flour, hair and following powder samples: pork bones, human tooth, gallstone and kidney stone.

2.2. TXRF method

The total reflection X-ray measurements were performed with the Picofox spectrometer (Bruker). In this method the dry residuum of liquid sample is analyzed. The characteristic X-rays were excited in the samples by 30 W Mo anode X-ray tube operated at 50 kV with an electron current of 0.6 mA. The primary X-ray beam from the tube, monochromatized using the multilayer monochromator, was directed onto the studied sample below critical angle. The fluorescence X-rays from the samples were detected by Peltier-cooled XFlash® Silicon Drift Detector having an energy resolution ≈ 150 eV. The measurements were performed in the air. The Picofox allows to measure the characteristic X-rays of elements from Al to U (with exception of Zr to Ru). Spectrometer software (SPECTRA 7) allows both qualitative analysis of the spectrum and the quantitative analysis of the content of the sample. The lowest achieved value of detection limit, dependent on the kind of studied samples, the analyzed element, its concentration and measurement time, in presented experimental setup was on the level of single ppb (10^{-9} g/g). The main advantage of application of the TXRF method in elemental analysis of the medical and environmental samples is possibility of trace elements concentration determination.

TXRF measurements were performed for following samples: hair, human tooth, gallstone, kidney stone, blood, serum, urine, parsley, carrot, cabbage, cereal, flour, pork bones, pork meat and fish.

2.3. XRPD method

X-ray powder diffraction measurements were performed in the Bragg–Brentano geometry using X'Pert Pro MPD diffractometer (PANalytical). This diffractometer is equipped with Cu anode 1.8 kW X-ray tube with linear exit window and PW3050/60 goniometer with an angular resolution of 0.001° . For X-rays diffracted on an analyzed sample the position sensitive silicon strip detector (X'Celerator) with dimensions 15×9 mm² and 128 strips was used. The detector speeds up the data collection by measuring simultaneously about 2° of 2θ . The

measurements were performed in the 2θ angular range from 5° to 70° . Typical measurement time of one full angular scan was about 30 min. The sample was rotated with one rotation time equal to 2 s, therefore shorter than measurement time of one angular position. Obtained diffractograms were analyzed qualitatively with Highscore 3.0e program using PDF-2 Release 2009 database of International Centre for Diffraction Data.

In the presented studies the diffractograms were measured for human tooth, gallstone, and kidney stone samples.

3. Samples preparation

Applied sample preparation procedure was different depending both on the analyzed sample and analytical technique used in the measurements.

For the WDXRF the pieces of samples analyzed in the presented studies were ground with the compact mill (MiniMill2) by application of the rotation speed 300 rpm and grinding time $t = 6$ min. The final fineness of this dry grinding was down to diameter $< 20 \mu\text{m}$ (depending on material). The ground sample were next measured in the powder form (mass from about 0.5 g to about 5 g) using special powder sample container (Fig. 1a) and in the case of sufficient sample amount (≈ 10 g) also analyzed in the form of the tablet (Fig. 1b and c). In the tablet formation process the sample was mixed with wax binder ($\text{C}_{18}\text{H}_{36}\text{O}_2\text{N}_2$), whose mass was about 10% of the sample mass.

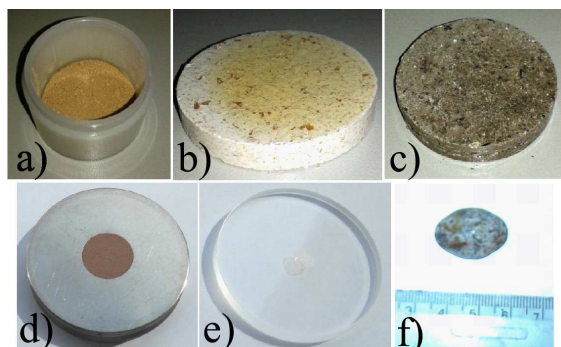


Fig. 1. Pictures of the samples prepared to the measurements: (a) powder sample of the gallstone measured by the WDXRF technique, (b) and (c) tablets analyzed by WDXRF, respectively slag and cereal samples, (d) gallstone sample in the sample holder prepared for XRPD analysis, (e) dry residuum of serum sample deposited on silicon backing and prepared for TXRF analysis. Picture (f) presents the gallstone analyzed in the presented paper using WDXRF, TXRF, and XRPD techniques.

Applied time of grinding and used pelletizing pressure (30 s at 20 tons) reduced the grain size effect and finally the constant X-ray intensity was observed.

For X-ray powder diffraction measurements part of the ground powder was placed in special sample holder (Fig. 1d) in which the sample can be packed without pre-

ferred orientation in whole volume being main assumption of the XRPD technique.

The TXRF measurements need the sample in the liquid form. Liquid samples were measured directly after adding Ga internal standard (750 μl serum + 50 μl Ga (100 $\mu\text{g/g}$), 3.3 ml urine + 1 ml HNO_3 + 0.5 ml Ga (10 $\mu\text{g/g}$) while solid samples in the amount of 0.2–0.3 g were mineralized with 4 ml of high purity HNO_3 and 0.1 ml of 100 $\mu\text{g/g}$ Ga standard (or 6 ml of HNO_3 and 0.2 ml Ga (100 $\mu\text{g/g}$)). The sample was further mineralized in microwave digestion system. Next, 5 μl of solution was pipetted into Synsil backing, and this drop was dried in infrared. The dry residuum (Fig. 1e) was next analyzed in Picofox spectrometer.

Part (f) of Fig. 1 presents the picture of the human gallstone, whose X-ray diffraction and elemental analysis was chosen to more detailed presentation in the paper.

4. Results and discussion

Figure 2 shows WDXRF spectrum measured for gallstone sample. The characteristic X-rays were excited in the sample by the primary X-rays generated in a Rh-anode X-ray tube operated, in presented scan, with voltage $U = 24$ kV and current $I = 100$ mA. The presented spectrum, corresponding to one of the measurement program scan, was measured in energy range from 2.0 keV to 2.7 keV applying Ge (111) crystal ($2d = 0.6532$ nm). Measured energy range corresponds with energy of the characteristics X-rays of the K series (P, S, Cl) and L series (Zr, Rh). Rhodium lines come from primary X-ray beam. Worth noting is very good lines separation in the spectra measured with WDXRF technique that make possible analysis of the samples rich in content. In the case of gallstone sample, for example, an application of the WDXRF method made it possible to determine the concentration of zirconium. That was impossible in TXRF measurement (Fig. 3). The TXRF spectrum is characterized by much worst energy resolution that is clearly seen comparing the energy range from 2.0 to 2.7 keV in Fig. 2 and Fig. 3. In the WDXRF scan also Zr K_α line is very well determined while on the TXRF is invisible due to the strong signal of Mo K_α line (from X-ray tube). Overlap of the measured lines is limitation of TXRF technique but in the case of trace element concentration determination this technique is much more effective than WDXRF whose detection limit is 10 $\mu\text{g/g}$. Consequently both methods are complementary for elemental analysis of the medical and environmental samples.

The TXRF spectrum presented in Fig. 3 for gallstone sample covers the energy range from 1.5 keV to 18 keV that corresponds fluorescence X-rays of the following elements: P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr (K_α lines) and Pb (L lines). Time of TXRF measurement was 1 h. Gallium K lines visible in the spectrum come from element added as an internal standard. Additionally, because of performing measurements in air, the Ar K lines are also observed. The Si K_α signal comes mainly from the silicon backing on which analyzed sam-

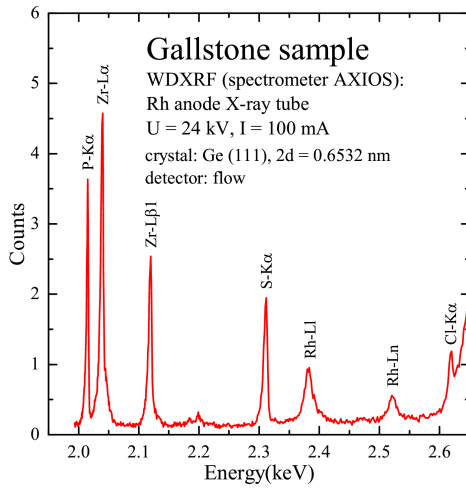


Fig. 2. An example of WDXRF spectrum of gallstone sample excited by the primary X-rays generated in a Rh-anode X-ray tube operated with voltage $U = 24$ kV and current $I = 100$ mA. The presented spectrum was measured in energy range from 2.0 keV to 2.7 keV applying Ge (111) crystal ($2d = 0.6532$ nm). The characteristic X-rays were detected by flow counter.

ple is placed. The characteristic X-rays of the elements lighter than silicon ($Z = 14$) are attenuated in air. Detection limit for the rest of elements observed in the spectrum was in the order of a few ng/g which allows determination of element undetectable with WDXRF method.

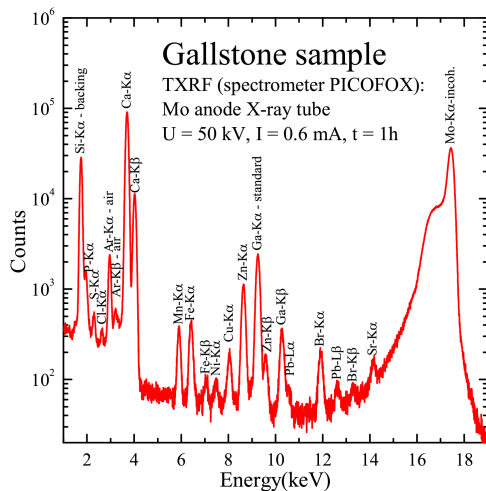


Fig. 3. An example of TXRF spectrum presented for gallstone sample excited by the primary X-rays generated in Mo-anode X-ray tube operated with voltage $U = 50$ kV and current $I = 0.6$ mA. The measurement time was 1 h. The gallium X-ray K lines are from the internal standard added for the calibration purpose.

Figures 4 and 5 present spectra of the liquid human biological samples, respectively: serum and urine (the measurement conditions like for gallstone sample). It is

worth noting that the peaks of Mn (0.037 ppm) and Pb (0.04 ppm) for serum and peaks of Sr (0.004 ppm), Cu (0.029 ppm), and Pb (0.074 ppm) obtained for urine, are close to detection limit of the TXRF technique.

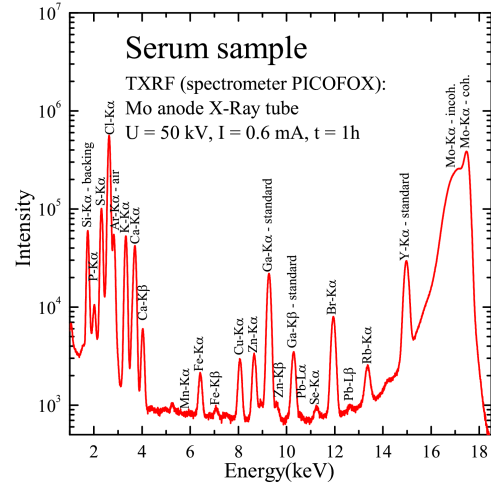


Fig. 4. An example of TXRF spectrum presented for serum sample excited by the primary X-rays generated in Mo-anode X-ray tube operated with voltage $U = 50$ kV and current $I = 0.6$ mA. The measurement time was 1 h. The gallium X-ray K lines are from the internal standard added for the calibration purpose.

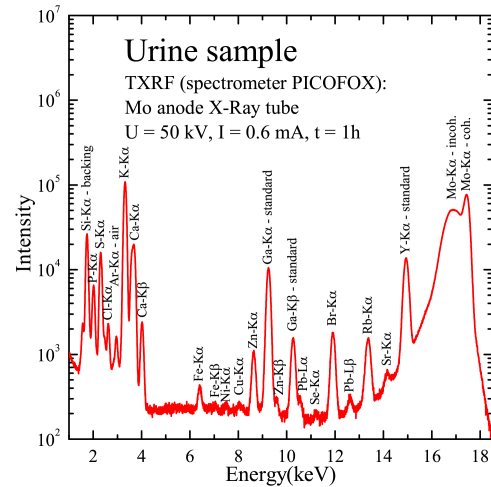


Fig. 5. An example of TXRF spectrum presented for urine sample excited by the primary X-rays generated in Mo-anode X-ray tube operated with voltage $U = 50$ kV and current $I = 0.6$ mA. The measurement time was 1 h. The gallium X-ray K lines are from the internal standard added for the calibration purpose.

The results of the elemental analysis of medical and environmental samples analyzed using WDXRF and TXRF methods together with experimental uncertainties are collected in Table I.

TABLE I

Concentrations of the elements in environmental and human medical samples determined by WDXRF and TXRF methods. In the case of the slag sample in the elemental analysis only WDXRF method was applied. In the table also experimental uncertainties are included.

Element concentration [%] WDXRF	Environmental samples				Human medical samples			
	slag	cereal	flour	pork bones	hair	human tooth	gallstone	kidney stone
O	–	36.7 ± 0.63	37.4 ± 0.64	32.5 ± 1.9	13.0 ± 0.43	57.6 ± 3.7	40.4 ± 3.9	26.3 ± 3.3
Na	1.64 ± 0.04	–	–	0.522 ± 0.09	0.051 ± 0.007	0.842 ± 0.140	–	0.117 ± 0.047
Mg	1.67 ± 0.04	0.042 ± 0.006	0.017 ± 0.004	1.28 ± 0.14	0.022 ± 0.004	2.36 ± 0.24	0.036 ± 0.024	0.179 ± 0.071
Al	10.7 ± 0.10	0.029 ± 0.005	0.0057 ± 0.0023	0.0071 ± 0.0044	0.012 ± 0.003	0.081 ± 0.013	0.0024 ± 0.0022	0.0058 ± 0.0044
Si	11.0 ± 0.10	0.050 ± 0.007	0.0090 ± 0.0028	0.039 ± 0.008	0.029 ± 0.005	2.22 ± 0.07	0.0083 ± 0.0042	0.015 ± 0.006
P	0.617 ± 0.024	0.163 ± 0.012	0.083 ± 0.009	7.22 ± 0.09	0.014 ± 0.004	13.4 ± 0.14	0.028 ± 0.007	1.12 ± 0.04
S	3.57 ± 0.06	0.148 ± 0.012	0.122 ± 0.011	0.147 ± 0.012	3.70 ± 0.06	0.059 ± 0.009	0.012 ± 0.005	0.023 ± 0.006
Cl	0.212 ± 0.014	0.067 ± 0.008	0.074 ± 0.008	0.118 ± 0.011	0.0082 ± 0.0030	0.208 ± 0.017	0.014 ± 0.045	0.035 ± 0.008
K	2.03 ± 0.04	0.286 ± 0.016	0.104 ± 0.010	0.138 ± 0.010	0.0015 ± 0.0010	0.021 ± 0.004	0.0031 ± 0.0022	0.011 ± 0.003
Ca	8.31 ± 0.09	0.037 ± 0.006	0.014 ± 0.004	14.8 ± 0.11	0.138 ± 0.011	23.8 ± 0.15	1.98 ± 0.04	10.4 ± 0.10
I	–	–	–	–	–	0.033 ± 0.005	–	–
Ba	0.067 ± 0.008	–	–	–	–	0.094 ± 0.009	–	–
Zr	0.024 ± 0.005	0.218 ± 0.014	–	0.440 ± 0.018	–	1.21 ± 0.03	0.206 ± 0.014	–
[µg/g] TXRF								
Ti	–	18.9 ± 0.46	9.46 ± 0.06	10.6 ± 0.88	20.0 ± 0.49	–	2.10 ± 0.51	–
Mn	–	25.3 ± 1.4	2.75 ± 0.37	0.940 ± 0.509	0.941 ± 0.485	–	43.6 ± 1.0	–
Fe	–	77.2 ± 3.5	16.9 ± 0.12	89.9 ± 1.2	146 ± 7.4	38.0 ± 0.31	31.8 ± 0.33	54.0 ± 2.8
Ni	–	0.550 ± 0.099	–	–	1.18 ± 0.08	–	0.690 ± 0.093	–
Cu	–	53.2 ± 1.4	0.270 ± 0.092	–	18.9 ± 0.06	–	8.82 ± 0.08	46.8 ± 1.5
Zn	–	32.6 ± 0.11	3.94 ± 0.29	190 ± 8.2	128 ± 6.0	21760 ± 1670	57.3 ± 0.21	445 ± 20
Br	–	1.80 ± 0.04	0.650 ± 0.102	1.18 ± 0.08	4.00 ± 0.20	–	213 ± 4	4.15 ± 0.21
Rb	–	2.10 ± 0.16	–	–	0.549 ± 0.101	0.870 ± 0.409	–	–
Sr	–	0.510 ± 0.095	–	61.7 ± 3.1	–	57.9 ± 5.0	2.69 ± 0.10	49.8 ± 2.6
Pb	–	3.10 ± 0.16	0.190 ± 0.081	1.65 ± 0.22	3.68 ± 0.20	26.5 ± 1.9	1.93 ± 0.30	8.79 ± 0.07

TABLE II

Concentrations of the elements in environmental and human medical samples determined by TXRF method. In the table also experimental uncertainties are included.

Element concentration [µg/g] TXRF	Environmental samples					Human medical samples		
	parsley	carrot	cabbage	pork meat	fish	blood	serum	urine
P	430 ± 14	371 ± 15	582 ± 20	2472 ± 88	1164 ± 52	240 ± 14	123 ± 7.4	174 ± 8.3
S	250 ± 14	472 ± 25	433 ± 21	1897 ± 70	1149 ± 56	830 ± 46	804 ± 45	–
Cl	115 ± 0.63	107 ± 1.2	49.8 ± 2.1	554 ± 18	383 ± 15	2155 ± 120	3765 ± 207	12.6 ± 0.51
K	2761 ± 119	1120 ± 50	4297 ± 156	2827 ± 102	1924 ± 87	1486 ± 83	143 ± 7.8	500 ± 23
Ca	373 ± 2.4	482 ± 1.8	256 ± 1.8	300 ± 1.2	143 ± 0.95	52.3 ± 3.0	82.6 ± 4.6	56.4 ± 2.0
Ti	0.259 ± 0.030	2.35 ± 0.21	2.49 ± 0.20	0.586 ± 0.050	31.4 ± 2.8	–	–	–
Mn	5.11 ± 0.34	1.13 ± 0.10	1.85 ± 0.13	0.439 ± 0.080	–	–	0.037 ± 0.010	–
Fe	7.77 ± 0.65	8.29 ± 0.73	10.4 ± 1.1	35.7 ± 3.0	23.2 ± 1.8	516 ± 29	0.765 ± 0.049	0.174 ± 0.025
Ni	0.233 ± 0.043	–	–	–	–	–	–	0.011 ± 0.004
Cu	0.497 ± 0.040	0.497 ± 0.037	–	0.016 ± 0.006	–	0.757 ± 0.051	0.813 ± 0.050	0.029 ± 0.008
Zn	4.73 ± 0.49	8.58 ± 0.90	2.56 ± 0.31	41.3 ± 0.68	14.4 ± 1.3	7.53 ± 0.43	0.785 ± 0.048	0.479 ± 0.442
Se	–	–	–	–	–	0.078 ± 0.010	0.042 ± 0.005	0.004 ± 0.002
Br	1.62 ± 0.18	1.93 ± 0.20	–	0.532 ± 0.056	0.380 ± 0.051	1.64 ± 0.10	1.77 ± 0.10	0.642 ± 0.062
Rb	1.22 ± 0.09	0.480 ± 0.064	0.674 ± 0.045	2.64 ± 0.11	0.790 ± 0.069	2.31 ± 0.14	0.190 ± 0.014	0.444 ± 0.023
Sr	2.50 ± 0.15	1.48 ± 0.15	0.146 ± 0.027	0.147 ± 0.027	–	–	–	0.024 ± 0.004
Pb	0.130 ± 0.005	1.66 ± 0.11	–	0.089 ± 0.003	–	0.129 ± 0.015	0.040 ± 0.006	0.074 ± 0.005

Taking into account area of application of WDXRF and TXRF methods the concentration of elements from oxygen (O) to calcium (Ca), zirconium (Zr), iodine (I) and barium (Ba) presented in Table I were obtained with the WDXRF while the rest of elements with the TXRF method. In general, the following elements were determined in the samples: O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, Zr, I, Ba, and Pb.

Table II presents concentrations of elements in the samples analyzed only by TXRF method and the following elements were found: P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, and Pb.

The measured concentration range was from the lowest 0.004 µg/g (Se in urine — TXRF) to the highest value 57.6% (O in human tooth — WDXRF). Dashes in the tables denote the cases when given element was not determined.

In the TXRF measurement the final uncertainty includes both the sample preparation systematic errors (weight measurement, used solutions volume determination, accuracy of determination of the internal standard concentration) and random errors (counting statistics, generator and X-ray tube stability, equipment errors, factors of calibration curve). The systematic error for all measured samples was about 5%. The random errors, mainly due to, counting statistics, strongly depended on the element concentration in the sample. For concentrations higher than $0.05 \mu\text{g/g}$ the uncertainty is on the level 5–10% while for concentrations less than $0.05 \mu\text{g/g}$ the uncertainty is even up to 50% depending on the element atomic number.

The total analytical error of WDXRF analysis is equal to the sum of the variances of all the individual sources of error, random (mainly: counting statistics, generator and X-ray tube stability, equipment errors) and systematic (sample errors (preparation, absorption, enhancement, particle effects, calculation of the results), sampling errors (dependent on sample mass and particle size)) [12]. In WDXRF measurements instrumental (equipment) errors were generally very small ($\approx 0.1\%$). The sampling errors were reduced by taking of a representative sub-sample. The errors of sample weight measurement was negligible. Corrections for inter-element effects was made by using Omnian corrections and grain size effect was avoided by using suitable specimen preparation technique (fine grinding) that was checked in accuracy and precision measurements. Finally, the experimental uncertainty was determined mainly due to counting statistics and for major elements ($> 1\%$ concentration) is 2–5% and for minor elements ($\approx 0.2\%$ concentration) it is 10–15%. For elements with concentration $\approx 0.01\%$ the uncertainty is 10–20%, while for the lowest measured concentrations ($\approx 0.001\%$) it is about 50% (and for powder samples even more). Taking into account the latest observation the element concentration equal to 0.001% ($10 \mu\text{g/g}$) is practical limit of element detection of applied WDXRF technique.

The lower limits of detection (LLD) of analyzed elements have been calculated on the base of performed measurements. The level of detection limit depended on the kind of sample, on the analyzed element and its concentration, on the measuring time. The ranges of LLD for TXRF measurements were the following: (a) about $1 \mu\text{g/g}$ for P, S, Cl, (b) about $0.1 \mu\text{g/g}$ for K and Ca and (c) from 0.004 to $0.01 \mu\text{g/g}$ for the rest of elements. In the case of WDXRF measurements the ranges of lower limits of detection were respectively: (a) about $0.5 \mu\text{g/g}$ for elements whose concentration in the sample was about 0.1% and less, (b) about $1 \mu\text{g/g}$ for concentration 1% and (c) $10 \mu\text{g/g}$ for concentration about 5% (or more).

The experimental accuracy for WDXRF and TXRF methods was on the level of 5–20%. In detail, in order to calculate the accuracy of the TXRF technique the certified reference serum (control serum sample in which elements concentrations are 1.0 mg/L) were daily measured

and next the ratio of the difference between measured and certified values to the certified value was calculated. The assumed difference should be less than 15–20%. In opposite case the recalibration of instrument is performed. In the case of WDXRF technique the accuracy was determined in the measurements of the geochemical soil, till and human hair standard reference materials. The sample were ground in the same way as analyzed medical and environmental samples and next measured in the powder form (mass about 12 g) using special powder sample container and also analyzed in the form of the tablet. In the tablet formation process the sample was mixed with wax binder ($\text{C}_{18}\text{H}_{36}\text{O}_2\text{N}_2$) whose mass was about 10% of the SRM sample mass. The calculated ratio of the difference between measured and certified values to the certified value was in the range from 5% to 20% (for Na and O). The stability of the spectrometer is controlled using monitor sample dedicated for AXIOS spectrometer.

In order to calculate the precision of TXRF measurements the whole analysis of one sample (from sample preparation to final result) was repeated ten times. Next the mean value and standard deviation of mean value of determined concentrations was calculated. The precision, defined as a relative standard deviation, was checked in two ways — either repeatability (higher than 85%) and inter-laboratory reproducibility (discrepancy is up to 10%).

The precision of the WDXRF measurements was calculated similarly like for TXRF technique by repetition three times the whole analysis of one sample. The best value of precision was 0.1% for Al whose concentration in the sample was about 3% while the worst was 15% for Y whose concentration was about $10 \mu\text{g/g}$. Concluding, the precision strongly depends on the amount of element in the analyzed sample.

In general, the elements concentration determined in discussed medical and environmental samples can be useful for many different interdisciplinary studies. However, obtained values and possible sample-element correlations are not interpreted in this paper in detail only some more interesting observations are further discussed. First of all in the human tooth sample (and also pork bones) the P, Ca, Zr, Zn, and Sr concentrations are higher than for other samples (Table I). Additional, the human tooth was the only sample for which the concentration of iodine (I) was obtained. The main elements of gallstone and kidney stone samples is oxygen (O) and calcium (Ca). In gallstone also higher concentration of zirconium (Zr) while in kidney stone of phosphorus (P), sodium (Na) and magnesium (Mg) is observed (Table I). The very interesting result is observation of the high concentration of Zr in gallstone. For this element it is in fact known that the main source of Zr in the human diet are vegetable and animal fats and it is accumulated in the liver and gallbladder [1].

Table I shows also that the oxygen concentration was not found only in the case of the slag sample.

From Table II it is interesting to compare how the element concentrations change for different human fluids, especially for whole blood and its serum fraction. The concentrations of potassium (K), iron (Fe), zinc (Zn) and rubidium (Rb) in the blood sample are about ten times higher comparing with serum sample.

The element concentrations in environmental vegetable samples are on the comparable level. In the animal samples the concentrations of the P, S, Cl, Fe, and Zn are higher than in the vegetable samples.

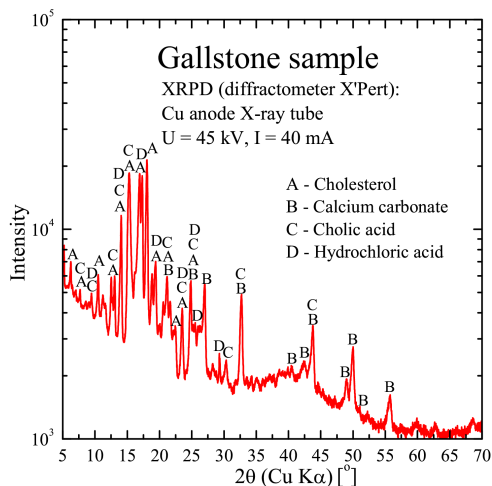


Fig. 6. Diffractogram of the gallstone sample obtained by XRPD method. The primary X-rays diffracted on the samples were generated in a Cu-anode X-ray tube operated with voltage $U = 45$ kV and current $I = 40$ mA. The compound obtained in spectrum: cholesterol (A), calcium carbonate (B), cholic acid (C), and hydrochloric acid (D).

TABLE III

Compounds determined in the human tooth, gallstone and kidney stone samples found by application of the XRPD method.

Human medical samples: compounds		
human tooth	gallstone	kidney stone
		weddellite ($\text{Ca}(\text{CO}_2)_2(\text{H}_2\text{O})$ 2.2)
	cholesterol ($\text{C}_{27}\text{H}_{46}\text{O}$)	weddellite ($\text{CaC}_2\text{O}_4(\text{H}_2\text{O})$ 2.375)
zinc oxide (ZnO)	calcium carbonate (CaCO_3)	whewellite ($\text{CaC}_2\text{O}_4(\text{H}_2\text{O})$)
hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$)	cholic acid ($\text{C}_{24}\text{H}_{40}\text{O}_5$)	whewellite ($\text{Ca}(\text{C}_2\text{O}_4)(\text{H}_2\text{O})$)
	hydrochloric acid (solution of hydrogen chloride (HCl) in water)	calcium oxide (CaO)
		sodium carbonate (Na_2CO_3)

The elemental analysis of the human tooth, gallstone and kidney stone samples were extended by X-ray powder diffraction measurements. As an example, experimental diffractogram of gallstone sample obtained by XRPD

method is presented in Fig. 6. The primary X-rays diffracted on the samples were generated in a Cu-anode X-ray tube operated with voltage $U = 45$ kV and current $I = 40$ mA. The compounds obtained in spectrum: cholesterol (A), calcium carbonate (B), cholic acid (C), and hydrochloric acid (D).

Table III summarized the information about compounds determined in the human tooth, gallstone and kidney stone samples found by application of the XRPD method. These results correspond to the elemental analysis of discussed samples performed by WDXRF technique. The main elements found there, respectively Zn, O, Ca, P for human tooth, O, Ca and Cl for gallstone, and O, Ca and Na for kidney stone sample, are components of the compounds recognized in the diffractograms.

5. Conclusions

Application of the complementary X-ray spectrometry techniques (WDXRF, TXRF and XRPD) allows for determination of chemical and elemental composition of medical and environmental samples. The chemical composition of the samples were found by XRPD technique with the detection limit on the level 1%. The WDXRF allowed for identification of elemental composition of the samples in the low- Z range and for elements with concentration higher than $10 \mu\text{g/g}$. TXRF measurements gave the information mainly about trace elements with concentration in the range of ng/g . As an example of the complementarity of these spectrometry techniques X-ray diffraction and elemental analysis of gallstone were presented in detail. The results make possible study of correlations between concentrations of elements in environment and human organism, which are in progress.

Acknowledgments

The equipment was purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovative Economy Operational Program (contract no. WNP-POIG.02.02.00-26-023/08).

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