TREATMENT OF HUMAN BODYFLUID SAMPLES FOR PHASE ANALYSIS

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Abstract

Bodyfluid analysis is a novel approach for researching environmental pollution impact on human health. Bodyfluids are in touch with outer environment mostly through the respiratory tract, and thus inhalation of polluted air may cause the penetration of suspended solid particles through biomembranes. Solid particles are able to penetrate biomembranes due to their size below 100 nm, therefore there is need for analytical methods, which are able to determine trace elements. Because of small amount of the obtained samples and potential infectivity of these samples, non-destructiveness of analytical method as well as the treatment of the samples is crucial. X-ray fluorescence spectroscopy (XRFS) is well known technique widely used for the determination of both major and trace elements in a wide range of materials. Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) and Raman microspectroscopy are also suitable methods for phase analysis of bodyfluids. The aim of the study is focused on comparison of experimental results from different human bodyfluids (mainly urine and amniotic fluid) treatment (various substrates) obtained by XRFS, SEM-EDS and Raman microspectroscopy.

Keywords

Metal-based particles, Human bodyfluid, X-ray fluorescence spectroscopy, Raman microspectroscopy

1. INTRODUCTION

The rapid rising of nanotechnologies with the production of different nanomaterials, including engineered nanoparticles and ultrafine particles, present a dilemma to regulators regarding hazard identification. Nanoparticles are commonly known as materials that have structural features with at least one dimension of 100 nanometers and less, such materials typically possess nanostructure dependent properties (e.g., chemical, mechanical, electrical, optical, magnetic, biological), which make them potentially risky to human health including the ability of entering the body and exhibiting biological and toxicological activity associated with their nanostructure [1]. The most probable pathway for general population exposure is direct inhalation of materials released into the air during their manufacturing [2]. However, ingestion and dermal exposure also cannot be neglected. The potential risk for human health concerns their ability to evade the natural defenses of the organism and to interact directly with proteins, enzymes, DNA etc. since they fall within the same size range. Experimental data reported that hazardous elements such as arsenic, lead, cadmium, and mercury are neurotoxic and may have teratogenic effects to human health [3 – 5].

Bodyfluids make an internal environment of tissues and organs and therefore bodyfluids composition can affect their functions. The amniotic fluid samples were chosen to show the invasiveness of submicron particles into the body and even more through the placental barrier and samples of urine as a marker of particles penetration into the body as well as the ability of the body to exclude those particles. Since bodyfluids are the biological material, they can potentially pose some biological risks for those who handle with the samples related to potential presence of infectious agents. The preferences of the methods for phase analysis are also crucial, because of the trace volume of analyzed elements. The aim of the study was to perform the evaluation of different bodyfluid samples treatment for phase analysis.

2. EXPERIMENTAL

2.1. Samples

Amniotic fluid samples were obtained as a residue after genetic analysis as a result for amniocentesis. The as-received samples were processed for further characterization in the Institute of Pathology, University Hospital Ostrava. For our experimental purposes the samples were (i) shaken and drop on a glass slide as multilayers and dried at normal temperature in laminar Flowboxu Steril-BIOBAN 48 Compact or (ii) centrifuged (JOUAN B4i, Jouan, France, St. Herblain) at 1500 rpm for 15 minutes and the sediment was subsequently dropped on a glass slide and dried at 37°C.

Urine samples were collected from workshop and research and development facility workers exposed to TiO_2 before and after a 8h shift by Pelclová and co-workers [6]. For our experimental purposes the samples were shaken and dropped on a glass slide as multilayers and dried at normal temperature in laminar Flowboxu Steril-BIOBAN 48 Compact.

2.2. Experimental methods utilized

Dried samples were analyzed using scanning electron microscopy (SEM Philips XL 30, Nederland) with X-ray microprobe of an Energy Dispersion Spectroscope (EDS). The samples were observed in the back scattered electron mode allowing visual detection of different elemental composition.

Phase analysis was performed by Raman microspectroscopy of all samples using Smart Raman Microscopy System XploRA™ (HORIBA Jobin Yvon, France). Raman spectra were acquired with 532 nm excitation laser source, and 1200 gr./mm grating.

The chemical compositions of the samples were determined by a SPECTRO XEPOS (Spectro, Germany) X-ray fluorescence analyzer (XRFS). The spectrometer uses 50W Pd X-ray tube, X-radiation is modified by secondary (Mo and Co) and polarization targets (highly oriented pyrolytic graphite and corundum). Measurements were performed in a helium atmosphere. The detector was a state-of-the-art silicon drift detector (SDD). X-LAB Pro software was used to control spectrometer functions and to evaluate data.

3. RESULTS AND DISSCUSION

Table 1 shows detected metals in the characterized samples. Since SEM-EDS and Raman microspectroscopy use visual selection of particles analyzed, the glass slide as a matrix is suitable for these purposes. The advantages of this matrix are costs, easy storage, easy manipulation and glass slides are good substrates for metal coating important for SEM-EDS analysis. XRFS is scanning some area, not only the selected solid particles, there is important role of the matrix, since glass slide contains many elements in bigger amount than analyzed samples, and therefore thus obtained results are not conclusive. Because of these findings, only few matrixes were used according to the previous experience with this method. Commonly used matrix for liquid samples for XRFS are different types of filters (analytical, diaphragmal, etc.). In case of bodyfluids, filters are not suitable, because of feathering the sample into bigger area and subsequent possible decreasing of amount of trace elements. As a result filters contain higher amount of trace elements than analyzed samples. The most promising matrix for XRFS seems to be the polypropylene thin film. Results of blank film are distinctively different than the results of the analyzed samples. Results of the bodyfluid samples on the polypropylene matrix are shown in table 1. The advantage of polypropylene

thin film is obtaining good results from XRFS, but big disadvantage is a complicated storage, which could lead to destroying a sample.

Table 1: Table of metals detected in the analyzed samples

Sample	Number of analyzed samples	Metals detected by SEM-EDS	Compounds detected by Raman microspectroscopy	Metals detected by XRFS*	Sample characteristic
Amniotic fluid	20	Al, Ba, Bi, Cr, Cu, Fe, Mn, Ni, Sb, Sn, Ti, Zn	$BaSO_4$, $BaCO_3$, Fe_3O_4, TiO_2	Al, Bi, Cu, Mn	Normal kareotype fetuses and also fetuses with congenital malformations
Urine**	60	Ba, Bi	$BaSO_4$, TiO_2	Al, Bi, Cr, Cu, Ni, Sr, Zn	Workers in plant producing nano-TiO_2, fluids provided before and after a shift

** only selected samples were analyzed by XRFS*
*** only selected samples were analyzed by SEM-EDS*

Because of small amount of the samples available for analysis there are some restrictions with testing of different matrixes. We had all samples dried on a glass slide, but only few samples were in sufficient amount in liquid state for multiple preparations. 3 samples of amniotic fluids and 3 samples of urine were dropped on different matrixes and the results obtained from those samples were compared. The results are discussed above.

Human amniotic fluid samples results obtained by SEM-EDS correspond with results obtained by Raman microspectroscopy as well as by XRFS, but the results obtained by Raman microspectroscopy do not correspond with the XRFS results. TiO_2, $BaSO_4$ and Fe_3O_4 were analyzed by Raman microspectroscopy, Ti, Ba and Fe elements were also detected by SEM-EDS. Example of Fe-based particle detected in human amniotic fluid is presented in figure 1.

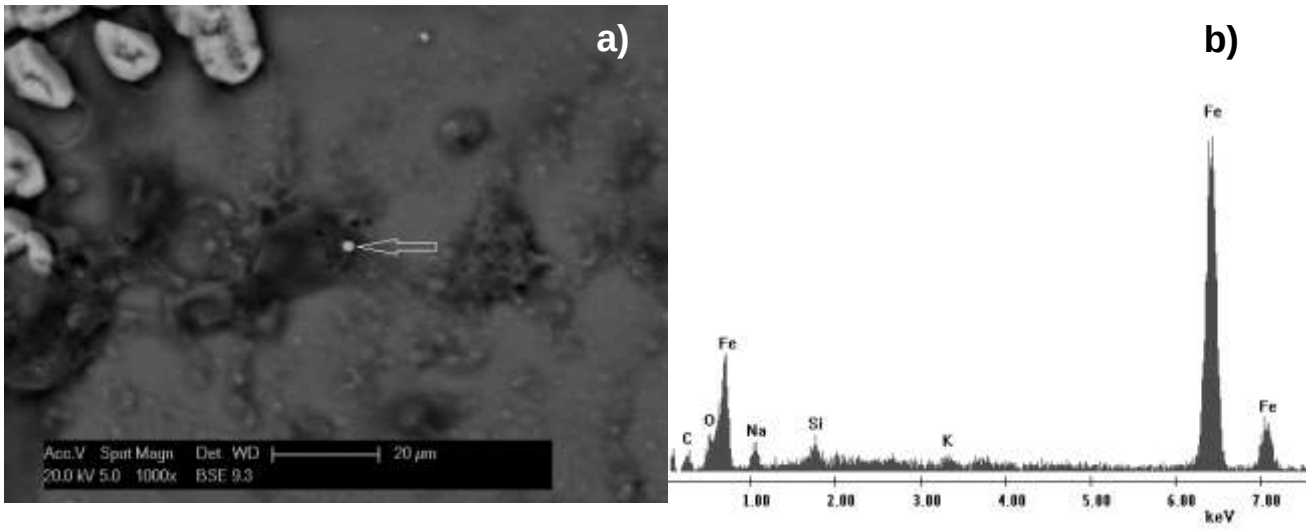

Fig.1: SEM image of Fe-based particle detected in human amniotic fluid (a) with corresponding EDS spectrum (b)

XRFS results for all the analyzed samples show that Ba and Ti elements were under the detection limit of the method in these samples and Fe amount was lower than the amount of Fe in the blank film. Results obtained from Raman microspectroscopy do not correspond with SEM-EDS, because there was no metal detected in urine samples by SEM-EDS. Raman spectrum of barite detected in urine is shown in figure 2.

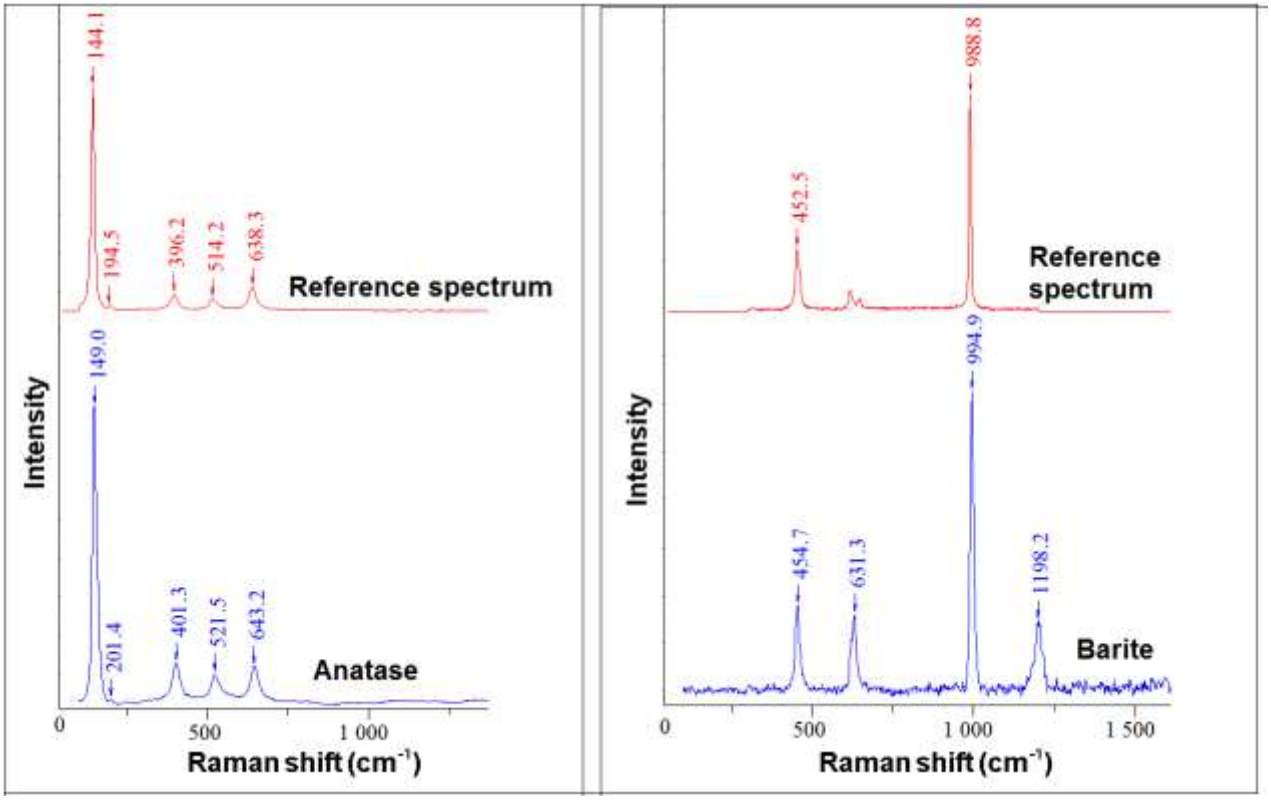

Fig.2: Raman spectra of anatase (left) and barite (right) detected in the urine samples

Raman microspectroscopy and SEM-EDS proved to be suitable methods for bodyfluid analysis as well as for human tissue samples which was proved by previous research [7], but there is a need for other methods in order to perform a comprehensive characterization of particles present. Because of small amounts of samples and present trace metals, the choice of the method is complicated and requires different treatments of the samples prior an analysis. For example, as shown in previous research [7] X-ray diffraction analysis was not able to detect metals in bodyfluids, because of the higher detection limit of this method.

4. CONCLUSIONS

Suitable bodyfluid sample treatment is drying the drops on different matrixes. For maximizing the signal achieved, the multiple dropping and subsequent drying was used. For Raman microspectroscopy and SEM-EDS is a glass slide the suitable matrix, for XRFS is the most suitable matrix was found to be a polypropylene thin film. The combination of the methods of human bodyfluid analysis it is important to get the complex information about bodyfluid composition. This information is need-to-know for determination of environmental effects on human health.

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