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Determination of chemical composition of individual airborne particles by SEM/EDX and micro-Raman spectrometry: a review

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Abstract. The strategies for sampling and analysis by SEM/EDX and micro-Raman spectrometry for individual airborne particles analysis as applied at the University of Antwerp (Belgium) by the MITAC group have been reviewed. Microbeam techniques provide detailed information concerning the origin, formation, transport, reactivity, transformation reactions and environmental impact of particulate matter. Moreover, some particles of certain chemical properties have been recognized as a threat for human health and cultural heritage objects. However, the small sizes of particles result in specific problems with respect to single particle analysis. Development of equipment and software for improvement of analysis and quantification are reported.

1. Introduction

Solid and liquid phase matter suspended in the atmosphere is addressed as ‘particulate matter’ (PM) or ‘aerosols’. PM consists of a mixture of many compounds, all in variable concentrations. The analysis of gaseous pollutants originally dominated the environmental air monitoring; nowadays, however, it is confirmed that atmospheric particles play a much larger role in environmental risk. It is now well understood that they are the major transport form of material in air, frequently contain toxic components and influence the global climate and the visibility of the atmosphere [1]. It is already a common knowledge in the field of cultural heritage and preventive conservation, that some particles (such as soot) are a threat for work arts due to soiling or blackening and constituting a medium for the adsorption and oxidation of several gases. S-rich material can cause discoloring of the paint by oxidation with H_2O to H_2SO_4; $(NH_4)_2SO_4$ can induce a bloom on varnish; $CaSO_4$ can cause blackening by adsorption of soot; Fe-rich particles play a catalytic role in the oxidation of SO_2 to H_2SO_4; etc. [2]. Without the tools suitable for single particle analysis it would be much more difficult to recognize the sources of the damage and to suggest remediation measures but also to take preventive actions. Last but not least, the impact of air quality on human health is now doubtless. Numerous studies have demonstrated associations between day-to-day variations in ambient particulate matter concentrations and day-to-day variations in health, including morbidity but also mortality [3]. Due to the highest annual mean PM concentration in Europe, Belgium has the highest estimated loss in life expectancy that can be attributed to PM, estimated to 15 months compared with 9 months as the European average [4]. The knowledge about the chemical nature of suspended particles would allow discriminating most hazardous ones and taking appropriate preventive actions.

It is a common strategy now in PM monitoring to perform bulk analyses; however, the atmospheric behavior of airborne particles is determined not only by their composition but also by their morphology. Since the atmospheric particles are chemically and morphologically heterogeneous, and the average composition and average aerodynamic diameter do not describe well their population, microbeam techniques facilitating single particle analysis must be applied. This type of analysis can be employed in studies dealing with important issues such as the source characterization and apportionment, particle transport and deposition or with various reaction mechanisms such as sulphatation and condensation [5]. However it requires a totally different analytical approach, starting from sampling methods through the whole measurement process. Due to small sizes of the particles, it is necessary first to collect and analyze a large number of particles to ensure the statistical relevance of the results. Nevertheless, much effort has been made so far to improve both the equipment and the compatible software [6-10].

This review presents mostly the research on individual airborne particles that has been done at the University of Antwerp (Belgium) by the MITAC group [11]. It includes about two decades of research aiming at single particle analysis, resulting in a number of articles, book chapters and conference presentations. The MiTAC group has a big contribution to the recognition of chemical properties of individual airborne particles worldwide; the home-made software designed by the MiTAC group enabled automatic, computer controlled measurement of particles along with subsequent numerical and statistical analysis. The effort to combine different microbeam techniques, such as electron and laser microprobe in order to obtain both elemental and molecular composition of single particles, has also proved to be successful.

2. Sampling of particulate matter for single particle analysis

Different sampling strategies of particulate matter for single particle analysis have been applied. The choice of the sampling strategy mainly depends on the desired information and/or limitations such as sampling conditions or sampling space. Generally the set up for sampling consist of a sampling unit, collecting either the total suspended particulate matter (TSP) or size segregated particles, a pumping unit and often a gas counter or flow controller**.** Size segregated sampling is by far the most widely applied technique in single particle analysis because it allows the separation of particles according to their cut-off diameters; however, in some cases TSP might be very useful, especially when aqueous particles (e.g. in rain) are to be analyzed.

2.1. Total Suspended Particulate matter (TSP)

Samples of single particles collected without size segregation, by means of filter units (e.g. on Nuclepore filters) may be of great importance, since due to the homogeneous distribution of particles on the filter, absolute numbers of collected particles can be calculated by extrapolation of the results of the analyzed part of the filter. This is not possible for the inhomogeneous impactor samples [12].

2.1.1. Air sampling

Total airborne individual particles were investigated in environmental issues such as analysis of the aerosols above the Lake of Baikal (Siberia) done by Van Malderen and co-workers [13] or in issues more directly connected to human health such as analysis of wood combustion particles emitted by industrial heating boilers during normal winter operation, as done by Osán et al. [14]. In both cases a

47 mm diameter, a 0.4 pore-size Nuclepore polycarbonate membrane filter (aerosol-grade) was used. The filter was placed in a plexi glass filter holder with a hat-type cover, to protect against rain or wet drops coming from waves. The cover was found to leave the aerosol size-distribution almost undisturbed [15]. The filter holder was connected to a vacuum pump, which was operated at a flow rate of approximately 50 l min^{-1}, and equipped with an automatic timer system and a flow meter. Samples were taken usually over a 2-h period (total sampling time), which resulted in a proper loading of the filter for single-particle analysis. After collection, the filters were stored in airtight Millipore Petri dishes.

2.1.2. TSP sampling from water

The seawater samples from the North Sea [16] or rain water above the North Sea [17] were firstly collected. Immediately after collection, the samples were filtered through 25-mm diameter aerosol-grade polycarbonate Nuclepore filters with 0.4-μm pore size by using a Sartorius SM 16306 25-mm glass vacuum filter holder with a 30-ml funnel and with glass frit filter support in case of rain water samples and 47-mm filters when sea water was used. To enhance the homogeneity of the particle distribution, a paper filter was placed beneath the Nuclepore filter during filtration. A suitable particle density for single particle analysis was obtained by filtering 10 ml samples from the two sampling sites close to the coast sites and 100 ml samples from the other sites. To remove crystallized salts, the filters were rinsed three times with de-ionized water. The filters were placed in petri dishes, air dried and stored in a deep-freezer until preparation for electron probe micro analysis (EPMA).

Another application where rain samples were used for single particle analysis were samples of the ‘Asian Dust’ that were prepared by depositing an aliquot of rainwater on a Ag foil using a micro-pipette, followed by drying the wet particles in air [18]. To asses the origin of the dust, CJ-1 (China Loess Certified Reference Material) and the local soil particles were ground to microscopic size using an agate mortar, suspended in distilled water, and deposited on the Ag foil in the same way as the Asian Dust particles. The EPMA results were then compared.

2.2. Size-segregated sampling

Various types of size-segregated impactors are commercially available; they vary starting from the number of stages and cut-off diameter of particles and ending with the dimensions of the whole sampler which are suitable for sampling in special places or conditions.

May cascade impactors (theoretical cut-offs 20, 8, 4, 2, 1 and 0.5 μm) were used inside of museum buildings, like the Sainsbury Centre for Visual Arts (Norwich, UK) with a flow rate of 20 l min^{-1} [19]. Apiezon-coated Nuclepore filters were used as collection substrates. Sampling times were up to 3 h for the impactor stages with the largest cut-off sizes. The collection substrates of the impactor stages with smaller cut-off sizes were retrieved after shorter sampling periods (7 to 30 min); since the number concentration of smaller particles in the air is larger than that of bigger particles, it is necessary to collect smaller particles in shorter sampling duration, in order to avoid creation of artificial agglomerates while overloading the sample. May cascade impactors were also used in the survey carried out to characterize the particulate matter in the Royal Museum of Fine Arts (Antwerp, Belgium) [12]. Sampling times ranged from a few minutes to a few hours, depending on the impactor stage. Apiezon-coated Nuclepore filters were also used as collection substrate.

Berner type impactors with 9 stages with cut off diameters of 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 μm were used for different types of aerosols e.g. for indoor sampling by Brimblecombe and co-workers [19] or for sampling of a particles above the North Sea [20]. The flow rate was 29 l min^{-1}. The particles were sampled on aluminium foil. Considering the cut-off diameters of the different stages, the time available for sampling and the size limit for the detection of particles by the electron probe microanalyzer (~0.2 μm), only six stages were selected for this sampling (stages 3–8). The sampling time varied between 2 (for stage 3) and 180 (for stage 8) min, to obtain the best loading of particles in the impacted spots. The collected samples were put in plastic carriers, sealed, and stored in a desiccator. The same type of impactor was used in Sainte Chapelle, Paris [21]. However here aerosol particles were sampled on Ag foil using four stages of a Berner impactor, with aerodynamic diameter cut-offs of 4, 2, 1 and 0.5 μm for stages 4, 5, 6 and 7, respectively, at a 20 l min^{-1} sampling flow. The sampling time ranged from 10 to 180 min. Spolnik and co-workers [22] used Si wafers and Ag foils as collection substrates for Berner-impactor sampling in churches that are valuable from a cultural heritage point of view, located in Italian Alps and southern Poland. The Si wafers were used to collect the finest particles (cut-off diameters of 0.25 and 0.5 μm, corresponding to stages 3 and 4, respectively). The particles collected with cut-off diameters of 1-8 μm (stages 5-8) were deposited on Ag foils.

Another (peculiar because of its small dimensions) type of impactor for sampling of particulates is Battelle 5–stage impactor (cut-off values 4, 2, 1, 0.5 and 0.25 μm) [12], used when sampling was to be done in limited spaces, e.g. interspaces between stained glass windows and protective glazing [22] or museum show-cases. The particles were collected on special transmission electron microscopy (TEM) grids made of a very thin carbon film (15–25 nm) supported by a copper mesh.

Nuclepore filters of 0.4 pore-size and cyclone sampling of particulate matter <2.5 μm, were used for collection of wood combustion particles emitted during normal winter operation of an industrial heating boiler [14].

De Miranda and co-workers [23] collected the aerosols in the Sao Paulo Metropolitan Area using stacked filter units (SFU) which separated the aerosol as coarse (2.5–10 μm equivalent aerodynamic diameter (EAD)) and fine fraction (<2.5 μm EAD) and cascade impactors of the MOUDI-type (‘‘micro orifice uniform deposit impactor’’, [24]) which does so in 10 different stages whose nominal 50% cut-off points (D50) are: after-filter, 0.093, 0.18, 0.33, 0.56, 1.0, 1.8, 3.2, 5.6 μm. The MOUDI impactor has rotating impaction plates and provides nearly uniform deposits over circular impaction areas. Nuclepore filters with 8 and 0.4 μm pore-size were used for the SFU. The MOUDI was operated with 8 μm pore-size Nuclepore filters (Teflon-backed filters with 37mm diameter) and have a 1 μm pore-size after-filter. A portable sampler called ‘Mini-Vol’ was also used to monitor particles with diameters below 10 μm (PM10). Again, 0.4 μm pore-size Nuclepore filters and MOUDI impactors were used.

3. Electron microprobe measurements

Electron probe X-ray micro analysis (EPMA or EPXMA) is a microanalytical method where X-ray radiation emitted by a sample exposed to an electron beam is detected. Since a variety of signals is available that can provide various information, this technique simultaneously detects elemental (qualitative) composition and morphology of analyzed objects, even for a small interacting volume such as a single atmospheric particle [1,15,16] The X-ray photons give information on the elemental composition while the signal derived from backscattered electrons can provide the information on the roughness (topographical image) and the atomic number variation with the location in the sample (compositional image) [5]. These features are definitely applied in scanning electron microcopy combined with an energy dispersive X-ray detection (SEM/EDX). What is then the difference between EPMA and SEM/EDX? These two are nowadays practically the same; only is SEM more conceived for imaging: larger electron focusing coils, detectors at a lower angle, X-rays have a longer path through the sample, hence it becomes less quantitative. In EPMA, it is reversed – some imaging capacity is sacrificed for better quantification: the X-ray detector is positioned at a larger angle from the sample, hence there are less matrix effects. And most often, a wavelength dispersive X-ray fluorescence (WDX) detector is (or can be) used. However, during the last decade, due to technological advances, the differences between SEM/EDX and EPMA have been reduced. In this review both terms will be used interchangeably.

Studying the particulates by single particle analysis with the use of EPMA requires, however, the measurement of large population set in order to obtain statistically meaningful data [5]. For this reason the possibility of automatic measurements by analyzing the back-scattered signal from particles mounted on a filter is one of the most advantageous features of the EPMA system, inevitable for representative individual particles analysis [16]. The automation improves the accuracy of the results by allowing characterization of a large number of particles in a reasonable time. Advanced home-made software was developed in our research group, to analyze individual particles in an automated way. The automated PRC (particle recognition and characterization) system uses the following methodology. The electron beam moves across the sample while the backscatter signal is monitored. In case the digitized backscatter signal exceeds a present threshold value, a particle is assumed to be detected. The coordinates of the contour points are determined and additional information such as particle diameter, perimeter and factor shape are calculated [25]. Afterwards an energy-dispersive X-ray spectrum is collected [26,27]. The intensities of the characteristic X-ray peaks present are stored together with the size information as an object vector for the individual particle. Next, the program turns into its search mode to localize and measure the next particle. Thus the PRC program is set up in three sequential steps: localizing, sizing and finally chemically characterizing the particle. Thanks to this software around 500 particles can be measured in less then 2.5 hours under optimized working conditions [5,26,27].

An important obstacle which had to be faced in EPMA or SEM analysis was the determination of low-Z elements such as carbon, nitrogen and oxygen, in addition to the elements ($Z \geq 11$) observed by the conventional EPMA technique. These elements have to be detected if an adequate identification of low-Z elements and chemical speciation at a single particle level needs to be done. Low-Z elements are very important because they form the major mass of aerosols. This is not possible by EPMA with conventional EDX detectors, mainly because the Be-window, which is used for protection of the semiconductor detector crystal, absorbs low-energy X-rays and thus prevents detection of the low-Z elements X-rays. By the application of the modified EPMA technique, which employs either a windowless or thin-window EDX detector [2,6,28], chemical compositions, including the low-Z components, of individual particles can be at least semi-quantitatively elucidated. The determination of low-Z elements in individual environmental particles allows us to profoundly improve the applicability of the single particle analysis; many environmentally important atmospheric particles, e.g. sulfates, nitrates, ammonium and carbonaceous particles, contain low-Z elements, which have not been characterized by the conventional EPMA.

Another problem of this technique is that it needs to operate in vacuum and samples are subjected to electron bombardment; in these conditions some loss or transformation of volatile or unstable compounds such as nitrates and ammonium, could occur between the sample introduction and the analysis step. To decrease the beam-damage effect, cold sample holder at the temperature of liquid nitrogen (-193ºC) was applied. This approach was found by Osán and co-workers [28] to be very efficient especially for larger particles as organic, nitrate and ammonium containing particles of diameter above 10 μm could be measured without significant damage.

3.1. Equipment

All applications reviewed here were carried out with the use of two instruments available at the Chemistry Department of the University of Antwerp. The first one is a JEOL JXA-733 (Tokyo, Japan), equipped with an OXFORD Link SATW ultrathin window energy-dispersive X-ray Si (Li) detector. The resolution of the detector is 133 eV for Mn Kα X-rays. The secondary-electron and backscattering-electron detector allow visualization and automated particle localization. The detector is equipped with a sub-micrometer thick polymer window. This apparatus is also connected to a TN-2000 X-ray analysis system, a self-modified version of a particle recognition and characterization program (Tracer Northern Middleton, WI, USA) that controls the automated analysis of the samples. The spectra are recorded by a CANBERRA S100 multichannel analyzer according to the corresponding energy [8]. All the information is stored on disk for off-line data processing. The estimated geometrical data are set as input parameters for the quantification procedure [8,29]. The vacuum system consists of oil rotary unit and oil diffusion pumps units. Detection limits for EPMA for most elements are between 100 and 1000 ppm.

Working parameters were as follows: an accelerating voltage of 25 kV [12,13,16,17], a beam current of 1 nA and 20s accumulation time for the X-ray spectra [12,13,14,16,17,21,22]. After introducing a thin-window detector, which made the determination of low-Z elements possible, the optimal experimental conditions ensuring a low background level in the spectra and a high sensitivity for light element determination, different parameters: a 10-kV accelerating voltage [8,14,20,21,22,29] and the beam current of 0.5 nA was applied [4,20,29]. In order to obtain statistically enough counts in the X-ray spectra and to minimize the beam damage effect on the sensitive particles, a typical measuring time of 20 s was used. Around 300-500 individual particles per sample were analyzed.

The cold stage of the electron microprobe allows the analysis of particulate samples at liquid nitrogen temperature and the measurements were carried out at approximately -193ºC stage temperature [8,21,22,29]. Another feature of this microprobe is the application of a continuous cleaned N_2 gas flow to reduce the deposition of the oil residues from the vacuum pumps.

The second apparatus is a SEM/EDX (JEOL-JSM 6300) unit equipped with an energy-dispersive (EDX) detector (Princeton Gamma Tech, NJ, USA), a backscattered electron (BSE) detector and a secondary electron (SE) detector. The Si(Li) detector can be operated in windowless mode for identification of low-Z elements and its resolution is 170 eV for Mn K_α X-rays [6]. Aerosol samples from the Correr Museum in Venice [2] and from Sao Paulo [23] were analyzed in the automated mode. In order to analyze as many particles as possible within a reasonable time), a software program was adapted in the laboratory from the commercially available 'integrated microanalyzer for imaging and X-rays' (IMIX) program (Princeton Gamma Tech) which locates the particles on the filter, determines their size, acquires BSE and SE images and performs EDX analysis within each particle. The intensities of the characteristic peaks in the spectra are determined by the top-hat filter method. Compared to JEOL JXA-733, image processing and mapping are more efficient owing to the newer data system.

3.2. Sample pre-treatment

The aerosol particles are usually collected on substrate material such as Al [20], Ag foil [21,22,29], Si substrates [22] or Nuclepore filters [14,17,23]. Nuclepore filters, although ideal for computer-controlled EPMA because of their microscopically flat surface, contain, however, carbon and oxygen; hence it is impossible to assess the concentration of these elements. Nuclepore filters are thus more suitable when information on heavier elements is desired. The use of this filters always require a conductive coating such as carbon coating or metallic coatings [6]. Both coatings have their negative sides; carbon coating introduces large inaccuracy in the determination of carbon in the particle and metallic coatings often overlap with characteristic lines of light elements, so that the accuracy of X-ray intensities of low-Z elements is very low. Aluminum foil, although it hinders the determination of Al, can be used for measurements of low-Z elements since it doesn't require any conductive coatings. Godoi et al [42] investigated various substrates (Ag and Al foils, Si wafers, Be discs, C tape and TEM grids) with regard to surface roughness, contribution to the EDX signal and stability under the electron beam. The topography (surface roughness) is an important parameter in the visual recognition of the particles in the secondary electron image (SEI) mode. As the results showed, the C tape could not be considered as a suitable substrate. Also Al foil appeared to have the roughness that resulted in difficulties in particle recognition. This seemed not be the case when Ag foil, Si wafers, Be discs and TEM grids was used. TEM grid appears to be superior in providing almost flat background and sharp analytical signal. Fairly goods results were achieved for Ag and Be line profiles. The contribution of the substrate to the quality of the X-ray spectrum was also investigated and the TEM grid again showed the best results.

To successively analyze the individual particles collected on a given substrate, a part of a substrate containing atmospheric aerosol particles needs to be glued to a Cu–Zn samples holder and, in case of Nuclepore filters, the sample is coated by a 25 nm carbon layer [14]. Some additional pretreatment was done e.g. by Osán and co-workers [14]. The collected cyclone fly-ash and bottom-ash particles were suspended in n-hexane, pipetted onto high-purity Ag foil and dried in air. No conductive coating was necessary for the thin-window EPMA measurements of these samples [14].

3.3. Fitting X-ray spectra

The final results obtained by EPMA, after electron beam – aerosols interaction, are stored as intensities of X-ray photons per energy channels, or, in the other words, as X-ray spectra. The analysis and deconvolution of X-ray spectra is done by the WinAxil software package, based on the internationally well-known AXIL (**A**nalysis of **X**-ray spectra by **I**terative **L**east Squares) fitting routines. A mathematical model is built using different parameters such as the background model (linear, exponential, Bremsstrahlung, smooth filter or orthogonal polynomial), the number of X-ray lines and the approximately correct values for the energy and resolution parameters of the spectrometer. The model parameters are then optimized by means of a non-linear least-squares procedure. The main goal of spectrum evaluation is to obtain unbiased, minimum variance estimates of the net intensities (under the peak area) of the fluorescence lines. Fitting an appropriate mathematical function to the spectral data can do this. The parameters of this function are chosen so that the weighted sum of squared differences between the measured spectral data y_i and the fitting function $y(i)$ are minimal. This object function is known as chi-square:

$$\chi^2 = \sum_{i=n_1}^{n_2} \frac{1}{w_i} \left[y(i) - y_i \right]^2 \tag{1}$$

The fitting function generally consists of two parts, one that describes the continuum and the other one that deals with the characteristic X-ray lines.

$$y(i) = y_{Cont}(i) + \sum_{j\ Elements} A_j \left[\sum_{k\ lines} R_{jk} P\left(i, E_{jk}\right) \right] \tag{2}$$

The inner summation runs over the number of K and/or L lines of each element with R_{jk} the relative contribution of line *k* of element *j*. The outer summation runs over all elements with the linear parameter A_j being the total intensity of element *j*. $P\left(i, E_{jk}\right)$ is a function that describes the shape of the X-ray line with energy E_{jk}. A typical implementation of $P\left(i, E_{jk}\right)$ consists of a Gaussian

$$P = G\left(i, E_{jk}\right) = \frac{Gain}{\sigma_{jk}\sqrt{2\pi}} \exp\left[-\frac{1}{2} \left(\frac{E(i) - E_{jk}}{\sigma_{jk}} \right)^2 \right] \tag{3}$$

in which the width and position obey the energy and resolution calibration functions of the spectrometer:

$$E(i) = Zero + Gain \times i \tag{4}$$

$$\sigma_{jk} = \left[\left(\frac{Noise}{2\sqrt{2\ln 2}} \right)^2 + \varepsilon\ Fano\ E_{jk} \right]^{1/2} \tag{5}$$

The fitting in the net peak area parameter A_j, but nonlinear in the parameters *Zero*, *Gain*, *Noise* and *Fano,* so that a nonlinear least squares procedure is required. In successive approximations, the values of the parameters are altered until the minimum in χ^2 is reached. A spectrum evaluation procedure based on these principles is very flexible since lines of one element can be grouped either as a multiplet for all lines of an element or separately for K_α and K_β lines. Escape and sum peak can be incorporated easily. Anyhow, the quality of the results depends highly on the accuracy of the model or the ability of the fitting function to describe the observed spectrum (figure 1).

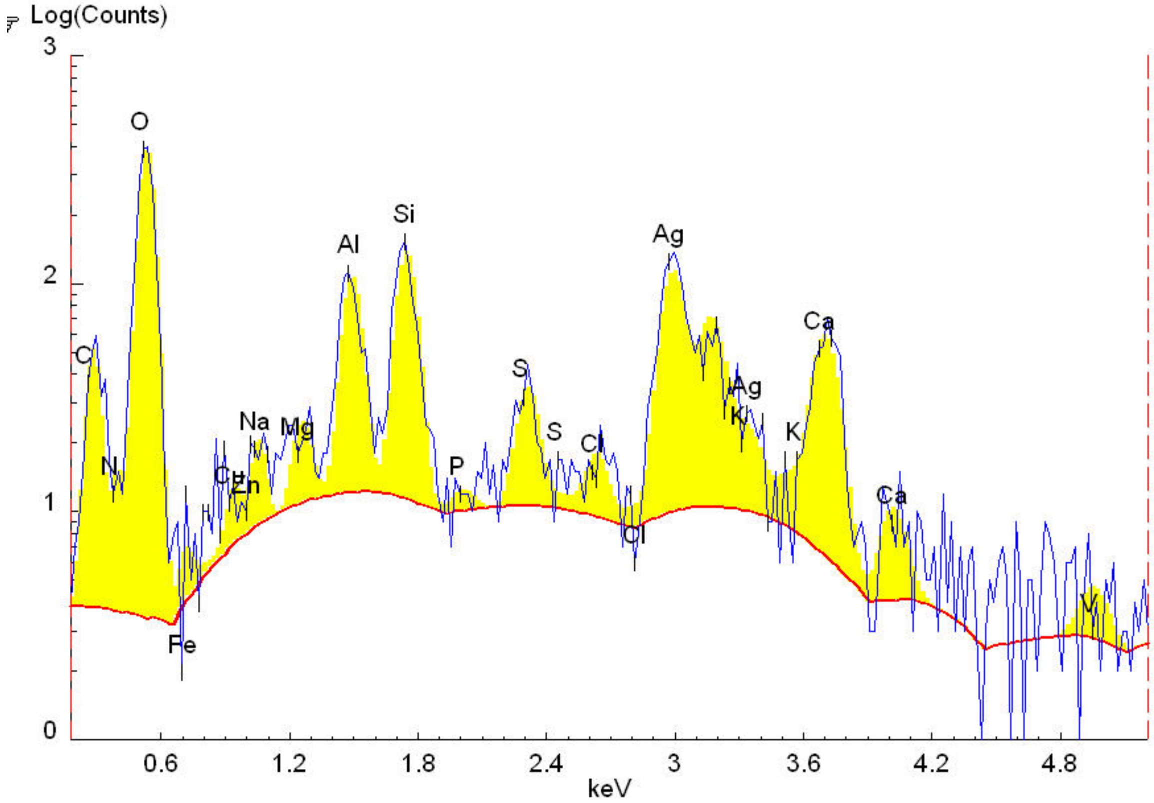

Figure 1. Typical X-ray spectrum fitted by WinAxil software

Two aspects of XRS hamper direct analytical use: matrix effects and spectral interference. By application of nonlinear least square fitting using Gauss or Voigt profiles modified with step and tail functions, overall spectra can be evaluated very accurately in a few seconds. The modification of Gaussian peak shape can be expressed by a mathematical model:

$$P\left(i,E_{jk}\right)=G\left(i,E_{jk}\right)+f_s S\left(i,E_{jk}\right)+f_t T\left(i,E_{jk}\right) \tag{6}$$

in which $G\left(i,E_{jk}\right)$ is the Gaussian as in equation (3) and f_s and f_t are (linear) parameters representing the fractions of photons that gives rise to a step and tail contribution. The step and tail functions can be written as follows:

$$S\left(i,E_{jk}\right)=\frac{Gain}{2E_{jk}}erfc\left[\frac{E\left(i\right)-E_{jk}}{\sqrt{2}\sigma}\right] \tag{7}$$

$$T\left(i,E_{jk}\right)=\frac{Gain}{2\gamma\sigma\exp\left[-\frac{1}{2\gamma^{2}}\right]}\exp\left[\frac{E\left(i\right)-E_{jk}}{\gamma\sigma}\right]erfc\left[\frac{E\left(i\right)-E_{jk}}{\sqrt{2}\sigma}+\frac{1}{\sqrt{2}\gamma}\right] \tag{8}$$

The functions are normalized, i.e. the integral of the function over the spectrum equals unity. The parameter γ is an extra nonlinear parameter, modeling the width of the tail. To keep the number of parameters to be estimated during the least squares fit as low as possible, f_s, f_t and γ are expressed as functions of the energy. This functional relation is fitted rather than the value for each peak individually. Spectrum evaluation based on this fitting model gives more reliable results than simple Gaussians.

4. Semi-quantitative microanalysis based on Monte Carlo calculations

The Monte-Carlo method uses the same physical parameters and processes as the fundamental parameter method but at an individual microscopic level to predict the intensities of characteristic lines. Different from the fundamental parameter approach, the Monte Carlo procedure is capable of dealing correctly with coherent and incoherent scattering phenomena. The data calculated by a Monte Carlo process can be considered as a spectrum seen by a detector with infinitely good resolution [30,31].

For the estimation of the quantitative composition of the microparticles, the size and shape of the particles and the matrix effect must be considered for light elements. It was found that the excitation interactions between electrons and the atoms of the matrix and the geometric effects of fluorescence signals for light elements in individual atmospheric microparticles can be described by Monte Carlo simulation [6]. This quantification procedure is based on a modified version of the single scattering CASINO Monte Carlo program [30,31], written in ANSI-C language, which was designed for low energy beam interaction to generate X-ray and electron signals. The modified version of the CASINO program allows the simulation of electron trajectories in spherical, hemispherical and hexahedral particles located on a flat substrate. The simulation procedure determines also the characteristic and continuous X-ray flux emitted from the substrate material and the influence of the substrate material on the energy distribution of the exciting electrons. This modified version of the CASINO program is the main module of the software package Elementary [20]. It consists of three main parts: Simulation, Quantification and Interpretation. A Monte Carlo calculation program is able to simulate electron-induced X-ray interactions within a single particle during scanning electron probe analysis. The same simulation can be used iteratively to quantify elemental concentrations starting from X-ray intensities. With the additional interpretation tools, Elementary offers a versatile, integrated software package for the fast interpretation of single particle analysis data.

5. Statistical approach

As a result of automatic SEM/EDX measurements followed by a fitting procedure and/or a simulation of elemental weight concentration, we receive a large database which needs to be reduced for analytical purposes. The variables used in the database are the particle number, element and – depending on the fitting method applied –either X-ray intensity (peak area) or weight concentration. The number of analyzed particles per sample ranges from ca. 100 to ca. 400 items, thus – in order to get an overview about the types of particles and element combinations – it is necessary to reduce the database size. In the field of single particle analysis, there are several major multivariate techniques: principal factor analysis (PFA) or principal component analysis (PCA), and hierarchical (HCA) or nonhierarchical clustering (NHCA). They can be applied independently or used in a combined approach.

5.1. Hierarchical Cluster Analysis

When dealing with a large number of particles, the first question is which particles were present in the sample [13]. The first task in multivariate analysis is to identify various particle types. This was done by a hierarchical clustering method. This approach has been proved a reliable method for classifying particles into groups according to their chemical composition. The most popular software used for HCA was the program IDAS [32]. The basics of HCA are settled in presenting every particle 'i' in N-dimensional space as an object vector with coordinates according to its N-elemental composition. Here N is the number of variables and equals the number of different elements analyzed in each sample. The method starts from m objects (particles) that are to be classified. At each step the two most similar objects (particles) or already formed clusters are merged into a single cluster. The similarity between two pairs of objects was estimated from the Euclidian distance coefficient between the objects. Ward's method (error sum of squares method) was used for the calculation of the distances between newly formed clusters and the remaining objects and/or clusters [34]. Each sample composed of a number of particles (100-400) must be subjected to HCA independently. The major problem with hierarchical clustering methods is to obtain the right number of clusters. This problem can be overcome by automated "cutting" of the dendrogram based on the Akaike's criterion. More detailed information on hierarchical clustering and the automated cutting can be found elsewhere [34,35,36].

Application of HCA to determine the type of particles caught in different environments appeared in the open literature devoted both to preventive conservation and cultural heritage protection. This aspect is emphasized in case of museums, galleries and churches where priceless artworks might be exposed to hazardous air pollutants. Owing to HCA applied to computer controlled (CC) EPMA measurements of PM collected in the Correr Museum in Venice (Italy), the particle types present in the indoor atmosphere were identified [2]. Ca-rich and Ca-Si-S-Cl-rich dominated the aerosol content (added abundance >50%) in the size range >1 μm in winter and in the entire investigated size range (0.5-20 μm) in summer. Aluminosilicates constituted a third group. Minor particle types were fresh sea salt (Na-Cl-rich), aged sea salt (Na-Cl-S-rich) and marine crystallization products, as well as Si-rich, Fe-rich and gypsum particles. Indoor air quality in the Sainsbury Center for Visual Artin Norwich, England, was also the aim of investigation by Brimblecombe et al. [19]. They distinguished several clusters of different chemical characteristics in the selected size ranges by means of HCA. It became clear that the smallest indoor particles were completely dominated by S-rich and low-Z element compounds (particles for which no elements with Z<11 could be detected in the X-ray spectrum). These particles could have a purely organic composition or they might consist of low-Z inorganic species, such as NH_4NO_3. In the larger size fractions (< 2 μm), aluminosilicates (mainly soil dust but also high temperature combustion fly ash) were dominating.

Gysels et al. applied HCA to characterize PM collected inside the Royal Museum of Fine Arts in Antwerp, Belgium [12]. In this case, the indoor size range ≥4 μm was characterized by Ca-rich, Ca–Si and aluminosilicate particles. The same types were identified in the size range 2–4 μm, but sea salt gained importance as well. The particles of the size below 2 μm were completely dominated by sea salt and aged sea salt. Ca-rich, Ca–Si particles and aluminosilicates became less important, while $CaSO_4$ and particles rich in S, Cl and Ca became more abundant. Fe-rich particles were present in every investigated size range, while low-Z, S- and Na-rich particles were only identified in the smallest size fraction. Similar particle types were identified in the outdoor samples; however, in comparison with the indoor samples, the sea salt abundance was much larger in the size fraction ≥2 μm and smaller in the size range <2 μm. The abundance of Ca-rich particles was significantly lower than indoors and Ca–Si particles were practically not detected. Fe-rich particles were present in every size range in higher abundances than indoors.

Particles attacking stained glass in the historical buildings were classified by Godoi and co-workers [21], e.g. in the Sainte Chapelle chapel in Paris. In total, all sampling implied the analysis of more than 10 000 particles. Owing to the HCA procedure, it was possible to divide coarse and fine PM fractions into the following clusters: SiO_2, AlSi, organic, $CaCO_3$, $CaSO_4$, FeOx, NaCl-dust, NaCl+$NaNO_3$ and P+dust in the inside and outside air; SiO_2, AlSi, organic, $CaCO_3$, $CaSO_4$, FeOx, $NaNO_3$-NaKCl, organic-dust-NaCl in the interspace between the deplaced original and the new window panes. The main difference between these was the relative abundance of each type. Three types of particles that are potentially dangerous to the glass were found inside and in the interspace; however all particle types were comparable and therefore deterioration cannot be indicated. The predominant presence of $CaSO_4$ is due to the conversion of $CaCO_3$ to $CaSO_4$ via reaction with SO_2 inside of this chapel, but probably even more due to the deterioration of the plastered walls [21].

The composition of suspended PM expressed as relative abundances of various particles (clusters) gets also a special attention due to its health impact. Low-Z EPMA facilitated measurements of C-rich particles together with other elements for Z less than 11. Osán and co-workers investigated wood combustion particles, most of which fall into the inhalable fraction [14]. Their HCA classification of particles based on EPMA measurements appeared to be an efficient tool to identify the origin of fly ash and to trace the emitted particles in the ambient air. Since the majority of the particles emitted from the wood burning plant studied in that work were found to be in the respirable size range, they can cause significant damage to the human health. The health aspect of PM composition was also the aim of aerosol characterization carried out in the Sao Paulo Metropolitan Area [23]. Using hierarchical cluster analysis on the automated EPMA data, particles could be identified and grouped into Si-rich particles, metals and metal compounds, sulphates, Ca-rich particles, chlorides, organic and biogenic particles. The particles derived from the soil appeared in the coarse fraction, Ca-rich particles and chlorides appeared with intermediate diameters, and organics and sulphates were present in the fine fraction.

5.2. Non Hierarchical Cluster Analysis

After the identification of the various particle types present in each sample, the next question was to compare the aerosol composition of all the collected samples. The global interpretation over a whole sampling campaign is very difficult when using only results of a hierarchical clustering on the separate samples. Gathering all particles in all the samples from one or more sampling campaigns creates a huge database which again requires data reduction. This can be done by a classification of all examined particles in similar samples together. It is not possible by HCA but can be done using a nearest-centroid-sorting technique [13,38]. Nearest-centroid sorting is a group of nonhierarchical clustering methods, which classify objects (particles) in clusters according to their distance from the centroid of a preset training vector. The distance between every particle and the training vector (centroid) is calculated and the particles which were incorrectly classified the first time are relocated. This procedure is repeated until convergence occurs [13,38].

The sequence of double HCA followed by NHCA based on the Forgy algorithm [37] was used in numerous manuscripts devoted to single airborne particles. This way of data reduction was successfully applied by Szaloki et al. [8] to characterize aerosol particles from the North Sea. This is a fundamental work describing the way of simulating quantitative composition of a particle together with comparison of real and simulated concentrations of simple chemical compounds. Then it was applied to real aerosols from the North Sea. The presented evaluation method was applied to analyze the light element content of aerosol particles collected over the North Sea. Elemental concentrations were calculated for each individual particle. To obtain information on the possible sources of the aerosol and the possible chemical interactions between gaseous and particulate pollutants, the particles were classified into 10 groups using the NHCA of Forgy [37] for the chemical and morphological data obtained by EPMA. The initial centroids for the method were obtained by two steps of HCA carried out for each sample. As light element analysis is possible by the present method, the nitrate particle type can be distinguished. The high abundance of organic-type particles can be recognized during the sampling period. The high abundance of sea salt particles in one is a fingerprint of marine origin. Ammonium-sulfate-type particles were also observed as a possible fingerprint of sea salt gaseous sulfuric acid reaction. A large fraction of particles shows a characteristic continental origin since soil-type, fly ash, ammonium and nitrate, sulfur-containing organic and metal oxides are observed.

Wide-range investigations on the North Sea aerosols were carried out by de Hoog and co-workers [20]. The procedure of sequential clustering by HCA and NHCA described by Szaloki [8] as well as the software for estimation of elemental weight concentration was applied to particle classification. For the comparison of the sample sets collected at a different location and time, all particles for each impactor stage (size fraction) were classified into 13 groups. To simplify the graphical representation, some similar clusters were taken together into one group afterwards (e.g. several, slightly different clusters of aluminosilicates were considered as one group). The 13 groups were named according to major chemical species in the groups identified on the basis of elemental concentration data of the groups: $(NH_4)_2SO_4$, $(NH_4)_2SO_4+NH_4NO_3$, NaCl, NaCl+organic, $NaCl+NaNO_3$, $NaNO_3$, organic, organic+S, aluminosilicates, Fe_2O_3, oxides, $(Ca/Mg)(CO_3/NO_3/SO_4)$ and biogenic.

Spolnik and co-workers investigated suspended particulate matter composition depending on the heating system in a case study of old churches [22]. For comparison of the samples collected during the operation of the investigated types of heating inside different churches, all particles were classified using NHCA. Fine and coarse particles were separately treated. The particles with diameters from 0.25 to 1 μm (stages 3-5 of the Berner impactor) were considered as fine particles, and those with diameters from 2 to 8 μm (stages 6-8) were considered as coarse particles. The average relative abundance (fine and coarse) was calculated for each set. In this manner, not only a classification of all particles in a distinct number of particle types representative for the sample can be obtained, but an allocation of an abundance value to each particle type for every sample as well. An overall analysis of the most important particle types allows determining the main sources responsible for soiling and blackening of objects displayed inside the churches.

5.3. Factor Analysis and Principal Component Analysis

The last step of the EPMA data processing is to find a correlation between the sources of the different particle types and their abundances. This can be achieved through so-called Factor Analysis (FA) or Principal Component Analysis (PCA). In FA each original variable is affected by various factors and some of them are shared by other variables in the data set (so called common factors) and the others are not shared (unique factors) [38]. The objective of FA is to take p variables (elements) and find linear combinations to produce uncorrelated new variables (factors), which are ordered in decreasing order of importance, so that the first explains the largest amount of variance. The proper number of factors should be considerably less than the number of variables, but should, on the other hand, explain a large fraction of the total variance. Only factor loadings greater than three times their standard deviation are considered as statistically significant. More detailed information can be found elsewhere [39]. In PCA there are no assumptions about the variables' structure. There are new hypothetical variables defined, independent of each other [38].

Van Malderen and co-workers [13] presented a combined way of data reduction applied to a number of PM samples collected over the total area of the Lake Baikal (Russia). They applied data combination of multivariate techniques to 52 PM samples (in total for each, 400 particles were analyzed, i.e. 20 000 spectra all together). HCA enabled to distinguish fourteen different clusters such as "Org", "Si-rich", "Ca-S", "Fe-rich", "Al-Si-rich", etc. Their abundances, however, varied between the samples, so in order to compare different samples, NHCA was performed. By gathering the particles from the samples belonging to the same location, the authors characterized the population of certain clusters in selected locations. A final step in the interpretation was to find correlations between the occurring particle groups and simultaneously link them to their various sources. Based on FA, the correlations and anti-correlations between elements were established; such as high loadings for Al, Si, K, Ca, Ti and Fe and anti-correlated organic type can comprise crust sources (different minerals).

A similar approach was applied by Worobiec et al. [40] to classify particulate matter collected in the Amazon Basin. For comparison of the different aerosol samples, the particles were grouped as coarse fraction and fine fraction and subsequently classified by NHCA based on the Forgy algorithm [41]. The initial centroids were selected by a sequence of HCA performed with the IDAS program [32]. A NHCA was performed on the fine and coarse size fractions independently. In the coarse particles data set, 14 different particle types were identified: aluminosilicates, P–S–Cl–K-rich, Si-rich, low-Z-rich, K–P-rich, NaCl, aluminosilicates agglomerated with fresh sea salt, K-rich, K–S-rich, CaSO4, P-rich, Na–P–S–Cl-rich, Fe-rich and Ti-rich. In the fine fraction there were following clusters identified: aluminosilicates, Fe-rich aluminosilicates, Si-rich (quartz), S-rich, low-Z-rich, P–S-rich, S-rich aluminosilicates, Fe-rich, P-rich, S–Cl–K-rich, CaSO4, Ti-rich, Cl-rich and K-rich. To investigate which samples had a similar chemical composition, the results from the NHCA were used as input for a PCA. These results proved the soil dust (Al–Si–Fe–Ti-rich species) must have been adverted from Sahara desert and also exclude the group of oceanic origin. For the fine fraction, the positive loadings of the first component represent the African dust as the source, while the negative loadings indicate the Amazon Basin (local/regional) origin. It also suggests that only two sources should be considered for the fine size fraction.

6. Micro-Raman analysis

Statistical operation of reducing the databases produced by automated SEM/EDX analysis results in a number of clusters representing chemically similar particles. The most common description given to the clusters comes from the most abundant elements in the cluster. Therefore we can find Si-rich particles or Si-Al-rich particles, S-O-N-rich particles, etc. It would be more informative and more useful to get the actual chemical composition, especially when the cluster name might raise some doubts, i.e. "C-rich". In this group, elemental carbon/organic particles as well as carbonates could be included. Furthermore, Na-S-C-O combination can be derived from a mixture of sodium sulphate and sodium carbonate but also a mixture of sulphate and soot. The technique which provides a molecular image could make the clustering more conclusive. One of the solutions is micro-Raman spectrometry (MRS). This technique is commonly used in the fingerprinting of matter due to its characteristic inelastic scattering and therefore provides valuable information on the molecular structure.

MRS is specifically suitable for single particle analysis since it is derived from a combination of confocal microscopy and Raman spectrometry. A laser spot of a size between 0.5 and 5 µm gets only a small sample volume exposed, providing information on the molecular make-up of particulate matter as small as 500 nm [42]. Figure 2 shows the visible light image of an Ag foil covered with airborne particles; this is the image recorded by a digital camera built in the MRS system, synchronized with a microscope and the motorized stage. Owing to such construction, it is possible to select individual particles and spot a laser beam on each of them, followed by recording of the Raman activity. Next to the microscope image, there is a Raman spectrum of one airborne particle presented. It is composed of the shifts belonging to $NaNO_3$ and elemental carbon, quite usual composition of PM within the size range 1-2 µm.

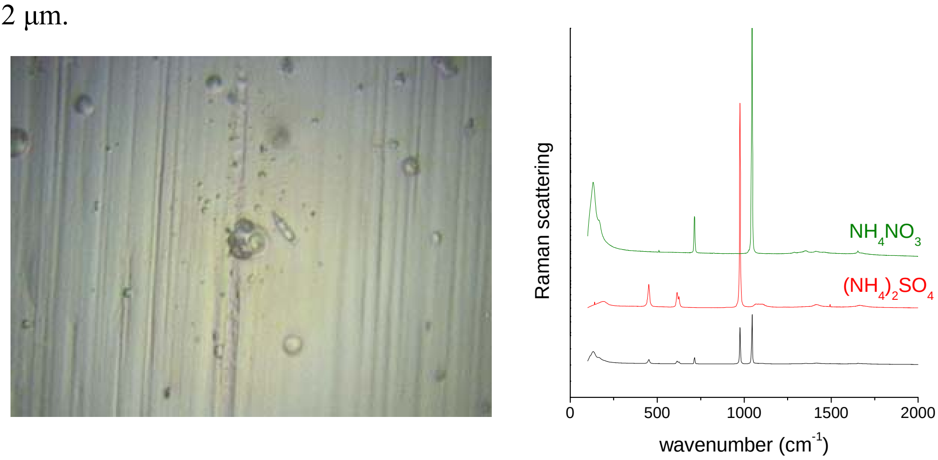

Figure 2. The visible light image of a silver foil covered with the airborne particles (magnification: 1000x) accompanied by a Raman spectrum of a particle (black line) consisted of ammonium nitrate and ammonium sulphate.

Application of micro-Raman analysis to particulate matter investigation has been the subject of some studies; however it was more adequate to geochemical research (soils, sands or sediments) instead of airborne particles [43]. However, some pioneering work on the possibilities of MRS towards analyzing individual particles was made by Potgieter and Van Grieken [44]. Nowadays MRS becomes more applicable in the field of aerosol analysis, although usually as an independent technique. Attempts to combine MRS with SEM/EDX or EPMA have also been reported [42,43]; however, the authors pointed out the limitations which have to be tackled while performing a parallel analysis with the two instruments together. Some of them are: different substrate requirements, lack of correlation between the probing depths, incompatible two images constructed in two different ways, limitations of magnification in case of optical image, carbon deposition during an electron beam scanning etc. Nevertheless, Godoi et al. described the results of parallel MRS and EPMA investigations of particulate matter collected in a museum (Rubens' house, Antwerp, Belgium). They selected several individual particles and compared their elemental composition given by low-Z EPMA, with their molecular make-up provided by MRS. C-rich particles were conclusively distinguished as soot or $CaCO_3$ or a mixture of both. The crucial issue for the two stand-alone instruments is relocation of the same particle. Once it is measured by SEM/EDX (EPMA), it has to be re-located in the optical image to be measured by MRS. Godoi et al. [42] reported TEM grids as a tool facilitating the particle relocation; TEM grids are suitable for both techniques (they are conductive and their Raman scattering is negligible) and they can create a sort of coordinate system inside which the particle position can be easily saved.

Another approach was applied by Worobiec et al. [45], who were investigating the PM composition in one of the world-famous museums - the Wawel Castle in Kraków (Poland). In this case the automated EPMA results were subjected to HCA and finally different clusters were recognized and verified by MRS. In this case two spots from the same Berner stage were analyzed independently by the two instruments, MRS and EPMA. Particulate matter sampled in Wawel Castle during the winter campaign appeared to be enormously rich with carbon. Its atmospheric origin (soot) was confirmed by MRS. Moreover; C-S-N-rich particles were recognized as a mixture of soot with ammonium sulphate, instead of organic or biogenic type.

7. Conclusion

For the last two decades, there has been extensive progress in the field of single particle analysis. It began with EPMA instrumental developments, followed by the design of computer software facilitating an automated mode of particle detection based on BSE images. Correlation of an electron beam scan with the digital image transformation, determination of the particle size from the projected area diameter and simultaneous recording of X-ray emission radiation created a new possibility of measuring a large number of individual particles in a fast automated way. The next step was the transformation of EDXRS spectra into a database correlated with abundances of elements in the particles. Since EPMA was equipped with a so-called atmospheric thin-window detector, it created a new possibility of investigating atmospheric particles composed of low-Z elements. Furthermore, development of new software for estimation of element weight concentrations (based on CASINO simulations) in each recognized particle enabled semi-quantitative analysis of single particulate matter. The first generation of this home-made software EPPROC was working in the text mode, but the next one – Elementary – was already equipped with the graphic interface. Since the atmospheric particles consist in a vast majority of low-Z elements, the chemical characterization of PM gained deeper insight. The final step of EPMA measurements is multivariate analysis leading to separation of clusters, i.e. particles of certain chemical similarity. Also in this stage the home-made programs (IDAS and Forgy) appeared irreplaceable. The whole procedure from sample preparation to recognition of the cluster types and abundances provided a lot of satisfying results about the atmospheric PM in ambient air over the whole world. The final stage of this methodological evolution is the combination of elemental composition and molecular analysis via interfacing two micro-beam techniques: EPMA and micro-Raman spectrometry. Application of both stand alone instruments and/or the hybrid version brought very promising perspectives for further single airborne particles research.

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