LASER RAMAN MICROPROBE
FOR THE CHARACTERIZATION OF RENAL LITHIASIS

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Summary. — Preliminary results are reported which were obtained by a laser Raman microprobe applied to the characterization of the microstructure of renal calculi and urinary sediments. This technique allows the direct analysis of the chemical and mineralogical composition coupled to a high spatial resolution. The results show the capabilities offered by this analytical technique in differentiating molecular species, namely oxalates and phosphates which have been evidenced in nephrolithiasis. The direct application of the microprobe on urinary sediments allows an early and rapid diagnosis of the risk level of patients.

Riassunto (Applicazione di una microonda laser Raman alle caratterizzazione della litiasi renale). — Vengono descritti i risultati preliminari ottenuti dall’applicazione di una microonda laser Raman alla caratterizzazione della microstruttura dei calcoli renali e dei sedimenti urinari. La tecnica permette l’analisi diretta della composizione chimica e mineralogica con elevata risoluzione spaziale. I risultati mostrano le possibilità offerte da questa tecnica analitica nella differenziazione di diverse specie molecolari, principalmente ossalati e fosfati, evidenziati nelle patologie nefrolitiche. L’applicazione diretta della microonda ai sedimenti urinari permette una rapida e precoce diagnosi del livello di rischio.

Introduction

Raman spectroscopy, because of its extreme sensitivity to molecular and crystal structure of the sample, has long been used to obtain useful information on the structure of the matter. Analytical applications of this technique have been however extremely limited by the small molecular Raman cross-section. Although the introduction of the laser as exciting source has increased the sensitivity of the technique, its direct application to the characterization of materials is still limited.

Very recently, by coupling an ordinary Raman spectrometer to an optical microscope, it has been possible to record the Raman spectra of individual microsamples because the small molecular cross-section is counterbalanced by the large irradiation density obtained when focusing the laser beam to a small pinpoint [1]. In this way, the analytical chemist can examine the molecular and crystal structure of matter according to the resolving power of the microscope which is used. The technique is therefore very effective for the non destructive analysis of individual particles opening new fields in science and technology [2].

The technique has the advantage of the ordinary Raman spectroscopy, namely: ease of sample preparation (in most instances the sample is analyzed as such), a response which is a function of the molecular and crystal structure for inorganic and organic substances. In addition, a morphological analysis of the sample is also possible making the Raman microprobe a challenging alternative to atomic microprobes which uses X-ray fluorescence analysis. This paper reports on the application of the laser Raman microspectrometry in the characterization of renal lithiasis.

Renal lithiasis is increasing in industrialized area due to a combination of several factors such as dietary habits, metabolic malfunctions and some alterations of the urinary tracts. It appears that adults and children as well suffer from this very noxious disease of the kidneys and, according to the opinion of most researchers, the mechanisms of stone formation is still a matter of speculation. Therefore, a precise analysis and/or characterization of renal calculi is a mandatory step in order to identify the pathogenic mechanisms which control the formation of the stones. For this reason a large number of analytical techniques are usually applied in order to characterize the chemical and mineralogic composition of the calculi.

However, calculi are organized in a very complex way which depends on the mechanism of formation, therefore a bulk analysis can only shed a little light on its microstructure. In order to have a better approach on the organization of the calculi, it is necessary to probe the stone on a smaller scale. The use of the micro-Raman spectrometry perfectly fits such a need because of its very large spatial resolution (about 1 μm). In
addition, the chemical compounds which are generally associated with the structure of the calculi can be easily found in the urinary sediments so that their characterization could be very effective in differentiating "high risk" from "low risk" patients.

Even in this circumstance the possibilities offered by the micro-Raman spectrometer, i.e. morphological analysis and direct chemical characterization, can be extremely useful.

**Instrumentation**

As said before, the Raman microprobe is an ordinary Raman spectrometer whose sample compartment has been replaced by an optical microscope. An argon ion laser, operating at a power level between 0.1 and 1 W is used for excitation. A prism premonochromator is used to filter out the laser plasma lines which can interfere with the Raman signals. The laser beam enters a slightly modified optical microscope and is focussed by means of the objective. The objective collects the photons backscattered by the sample which are analyzed on a double monochromator equipped with holographic gratings.

Light detection is performed with a cooled photomultiplier and a standard photon counting equipment. The resulting pulses are recorded by a counter controlled by a dedicated microprocessor which also controls the scanning motor and the slit width. In addition, the microprocessor performs simple calculations such as background subtraction, smoothing and storing of spectra on floppy disk for further retrieval. The spectra are presented in full colours on a monitor and can be hard-copied on a small line printer.

Particles are directly analyzed on standard microscope slides and they do not require special environments so that they can be analyzed in ambient air and in controlled atmospheres as well.

The performance of the instrument in sensing very small particles with a satisfactory S/N ratio is dependent on the particle size and the molecular cross-section of the material. For instance, particles such as titanium or lead oxides are well evidenced down to a size of 1 μm or less, while silicates, which usually exhibit small cross-sections, require particles of size larger than about 5 μm. Spectra of very small particles with small cross-sections can only be obtained with repetitive scans which can easily be handled by the microprocessor.

**Experimental**

The work carried out up to now mainly consists in a basic research toward the acquisition of a data bank concerning the Raman spectra of the compounds which have been found and positively identified in calculi and urinary sediments.

Figure 1 shows a spectrum which has been directly observed on a calculus removed from the upper urinary tract. The size of the stone was approximately 10 × 7 mm and only a small area was probed with the micro-Raman spectrometer. The spectrum identifies cystine as the major constituents of the calculus as it can be shown by a reference spectrum. The large Raman cross-section of the cystine allows a positive identification of this compounds in less than 5 min by just scanning the spectral region at about 500 cm⁻¹ and 2900-3000 cm⁻¹. The doublet in the latter region is to be assigned to N-H stretching modes.

Figure 2 shows the spectrum observed for a calculus which was previously identified as oxalate. The major constituent of an oxalate calculus is the calcium oxalate mono- and dihydrate which are known as whewellite and weddellite respectively. The two forms can be easily differential by Raman spectroscopy because the dihydrate species gives a very intense peak at about 1475 cm⁻¹ which splits in a doublet in the monohydrate form. The Raman splitting of these two peaks is about 25 cm⁻¹ which matches the observed splitting in Fig. 2, thus a positive identification of whewellite has been possible. It is worth noting that the weddellite has been identified in probing a calculus which was reco-
recognized as phosphatic in nature by bulk analysis. The characterization of oxalates is not very difficult owing to their relatively large cross-section. However most of oxalate calculi are fluorescent, therefore a very low signal to noise ratio is observed.

Other very important constituents of the calculi are the phosphate salts. Direct chemical and crystallographic X-ray analysis identifies calcium and magnesium phosphates as the major constituents of calculi, but unfortunately they cannot detect very low amounts of different species which can play a fundamental role in the formation and growth of the stone. For instance, has been recently suggested that nucleation of oxalic stone takes place on calcium hydrogen phosphate [3]. Figures 3a to 3c show that the three forms of calcium phosphates can easily be differentiated. All spectra show a relatively intense peak at about 960-970 cm\(^{-1}\) which is assigned to the symmetric stretching of the molecular ion \(\text{PO}_4^{3-}\) and weaker bands whose intensities and frequency shift can be positively used for the chemical identification.

Unfortunately, calcium phosphates have a very low Raman cross-section coupled to a very intense fluorescence and this causes quite a number of problems in order to sort out a well defined spectrum. Fluorescence can be eliminated by means of intense radiation with the laser beam at the maximum power level and several samples can be analyzed. For instance, Fig. 4 shows the spectrum of a calculus removed from a patient. It shows the presence of ammonium magnesium phosphate (Struvite) which has been identified by the intense peak at about 950 cm\(^{-1}\). It is worth stressing that the same spectrum has been found in several samples such as in the urinary sediments of the patient a few weeks after the surgical removal and in microspherules of size ranging from a few microns to several hundred microns which appear linked together in a chain-like appearance.

The major role of phosphates in the organization of calculi requires large efforts in order to obtain well defined and resolved spectra regardless of the fluorescence and the small cross-section. Low temperature ashing of the material can eliminate the organic matrix which is responsible for fluorescence, while the small cross-section can only be counterbalanced by the use of large power laser beams and highly sensitive electronics for detection.

Another constituent of the calculi is the calcium carbonate (Fig. 5). It is a constituent of biliar and pancreatic calculi in the form of calcite (trigonal) and aragonite (rhombic). These two species can be easily differentiated because their Raman spectra show an intense peak at 1090 cm\(^{-1}\) due to carbonate ion, a single peak at 710 cm\(^{-1}\) (calcite) which splits in a doublet in aragonite.

An additional class of substances which are present on some calculi is the purines. Although the work carried out up to now does not include studies on such substances, the laser Raman microspectrometry has been proved to be very effective in differentiating uric
The analysis of sediments is somewhat simpler than that of the calculi as such because in most instances the microcrystals are easily recognized by the morphological observation and the Raman spectrum is just used to confirm the presence of selected chemical species. The sediments are usually cleaned up with distilled water, dried and then analyzed. This procedure makes possible the direct observation of most common compounds found in sediments such as calcium oxalate and phosphate.

Conclusions

The use of the micro–Raman spectroscopy for the identification of selected chemical compounds on renal calculi has been proven to be very effective, mainly if the scientific interest is focussed on the microstructure or on the analysis of urinary sediments. The very large sensitivity, coupled to very good spatial resolution, is such that the technique seems to be very promising for understanding the chemical processes which control the formation of calculi.

Unfortunately, several problems have to be faced out. Indeed, a calculus is usually a very complex matrix where organic and inorganic components may be present in a very large variety of chemical species. Most of them are degraded when irradiated with intense laser beam or fluorescence confuses or masks Raman spectra. However, these difficulties can be overcome, namely in the field of urinary sediments in order to gain a method for the positive identification of normal or pathogenic urines [4]. In such a way an early diagnosis of renal lithiasis can be effectively done and the disease be controlled on time. Work on these aspects is now in progress.

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REFERENCES


REMOTE SENSING OF GASEOUS POLLUTANTS WITH LASER

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Summary. — LIDAR (LIght Detection And Ranging) and DIAL (DIfferential Absorption LIDAR) techniques make today possible remote measurements of pollutant gases and physical atmospheric parameters, in real time. With DIAL techniques pollutant concentrations and atmospheric temperature can be measured, with a spatial resolution of few meters, at distances up to few kilometers. A detailed description of two DIAL systems designed and realized in CISe laboratories is reported. A particular attention is put on the laser sources, the receiving optics, the detection devices and the data processing systems. Experimental results of the SO₂ concentration measurements in an area including a town and some industrial plants are reported.

Riassunto (Determinazione a distanza di inquinanti gassosi per mezzo di laser). — Le tecniche LIDAR (LIght Detection And Ranging) e DIAL (DIfferential Absorption LIDAR) rendono oggi possibile la determinazione a distanza di inquinanti gassosi e dei parametri atmosferici fisici, in tempo reale. Con le tecniche DIAL si possono misurare le concentrazioni di inquinanti e la temperatura atmosferica, con una risoluzione spaziale di pochi metri, fino a distanze di alcuni chilometri. E’ riportata una dettagliata descrizione di due sistemi DIAL progettati e realizzati nei laboratori del CISe. Particolare attenzione è stata posta alle sorgenti laser, ai sistemi ottici di rilevamento e di elaborazione dei dati. Sono riportati i risultati sperimentali della determinazione di SO₂ in una zona comprendente una città ed alcuni impianti industriali.

Introduction

Historically air pollution monitoring has been performed using chemical techniques and sampling for later laboratory analysis. Actually remote monitoring instrumentation, using laser, can provide three-dimensional, in situ, in real time and non intrusive pollution concentration measurements.

This method, named LIDAR (LIght Detection And Ranging) can provide the following types of measurements:

a) monitoring of the pollutant sources and concentrations over very large areas with high spatial resolution;

b) measurement of the transport of pollutants for validation of pollution transport models;

c) monitoring concentration of gases important for atmospheric photochemistry and physical atmospheric parameters like temperature and wind velocity measurements;

d) monitoring leakage from chemical plants and pipelines.

The main constituents for air quality management are aerosol and particles, oxides of carbon, sulfur compounds, nitrogen compounds, hydrocarbons and ozone; in the stratosphere it is very important to monitor also methane and chlorofluorocarbons for ozone photochemistry.

Principles of the LIDAR

LIDAR employs the back-scattered signal of a laser beam from the atmosphere to make measurements of the atmospheric properties by means of the scattering and absorption processes. It is generally used in a mo-
nostatic configuration in which the transmitter and the receiver are located at the same point. The utilization of laser sources permits many advantages for atmospheric diagnostics, because laser radiation can have a very narrow spectral width, the wavelength can be tunable and laser intensities can be generated in very short pulses. This last property is particularly useful because it can provide range information.

**LIDAR equation.** — A typical monostatic LIDAR configuration is shown in Fig. 1. A pulsed laser pulse is transmitted in a narrow beam with a coaxial receiving telescope that collects the radiation scattered in the backward direction. Neglecting multiple scattering, the back-scattered signal at wavelength is given by:

\[
P_2(R) = Q P_0 \frac{c \tau A}{2 R^2} \beta(R) \exp\left(-2 \int_0^R \alpha(r) \, dr\right)
\]

where:

- \(P(R)\) = optical power received from distance \(R\)
- \(Q\) = efficiency of telescope optics
- \(P_0\) = laser transmitted power
- \(c\) = light speed
- \(\tau\) = laser pulse duration
- \(A\) = telescope area
- \(\beta(R)\) = back-scattering coefficient at distance \(R\)
- \(\alpha(R)\) = extinction coefficient at distance \(R\)
- term \(\beta(R)\) is the sum of the back-scattering coefficients of Rayleigh and Mie
- term \(\alpha(R)\) is the sum:

\[
\alpha(R) = \alpha_R(R) + \alpha_M(R) + \alpha_{\text{GAS}} + \alpha_{\text{RAMAN}}
\]

where \(\alpha_R(R)\), \(\alpha_M(R)\), \(\alpha_{\text{RAMAN}}\) are the Rayleigh, Mie and Raman extinction coefficients and \(\alpha_{\text{GAS}}(R)\) is given by:

\[
\alpha_{\text{GAS}} = N(R) \sigma(\lambda)
\]

where \(N(R)\) is the pollutant gas concentration and \(\sigma(\lambda)\) is the absorption cross section at wavelength \((\lambda)\).

Because the laser pulse durations are about 10 ns and the typical beam divergence is \(10^{-3} - 10^{-4}\) rad it is possible to receive back-scattering from atmospheric volumes of the order of a few cubic meters at ranges of tens of kilometers.

In the LIDAR equation the information on the atmospheric properties are contained in the \(\beta\) and \(\alpha\) coefficient. \(\beta\) coefficient is due to the scattering contributions from all atmospheric constituents like molecules, aerosol, particles, water droplets and also includes both elastic and inelastic scattering processes.

The extinction coefficient \(\alpha\) includes all of the attenuation processes in the atmosphere; the cross sections of the various scattering processes range from values as high as \(10^{-8}\) cm\(^2\) s\(^{-1}\) for larger aerosol particles, down to \(10^{-28}\) cm\(^2\) s\(^{-1}\) for Raman scattering. \(\alpha\), \(\beta\) coefficients are not independent parameters and the general solution of LIDAR equation (1) is a complex problem.

**Dial techniques**

DIAL is acronym for Differential Absorption LIDAR which exploits the strong wavelength dependence of the absorption spectra of the atomic and molecular species in the atmosphere (Fig. 2).

A typical experimental set-up is shown in Fig. 3. In this method a tunable, two wavelengths laser is required. One wavelength (\(\lambda_{\text{on}}\)) is tuned to match a peak of an absorption line of the gas of interest while the second wavelength (\(\lambda_{\text{off}}\)) is tuned to a region of low absorption. Comparison of the two signals gives a direct measurement of the concentration of the pollutant.
The spatial resolution of the LIDAR is limited by the laser pulse duration and electronic bandwidth of the receiver.

The solution of (1) for $N(R)$, the concentration at distance $R$ on the range $R$, is given by [1]:

$$N(R) = \frac{1}{2\sigma \Delta R} \ln \left( \frac{P_r(\lambda_{on}, R)}{P_r(\lambda_{on}, R + \Delta R)} \right) -$$

$$- \ln \left( \frac{P_r(\lambda_{off}, R)}{P_r(\lambda_{off}, R + \Delta R)} \right) + B + T \quad (4)$$

where:

$$T = -2 \alpha \left[ (\lambda_{on}, R) - \alpha (\lambda_{off}, R) \right] \Delta R, \quad (5)$$

$$B = \ln \left[ \beta (\lambda_{on}, R + \Delta R) / \beta (\lambda_{on}, R) \right] -$$

$$- \ln \left[ \beta (\lambda_{off}, R + \Delta R) / \beta (\lambda_{off}, R) \right], \quad (6)$$

$$\sigma = \sigma (\lambda_{on}) - \sigma (\lambda_{off}). \quad (7)$$

Generally we can determine $N(R)$ only if we know the spectral dependence of $\alpha$ and $\beta$. However, if $\lambda_{on}$ and $\lambda_{off}$ are close, the terms $B$ and $T$ are nearly zero, so that we can neglect in the computation every parameter, apart $\sigma$. DIAL method can also be used with a topographic reflector (that is a wall, a bill, a tree) instead of the distributed reflector of aerosol and molecules.

In this case we do not obtain a range resolved concentration measurement but only the average concentration between the target and the DIAL system (Fig. 3). In this case the back-scattered laser energy is given by:

$$F_r = Q E_0 \frac{A}{R^2} \frac{\rho}{\pi} \left[ -2 J_0 R \alpha(t) \right] \quad (8)$$

where $\rho$ is the reflectivity of the topographic reflector and $E_0$ is the transmitted pulse energy. The average concentration is given by:

$$N = -\ln \left( \frac{Q(\lambda_{off}) E_0(\lambda_{on}) E_r(\lambda_{off})/2}{\sigma_L} \right) \quad (9)$$

where $Q(\lambda_{on})$ and $Q(\lambda_{off})$ are the optics efficiency at $\lambda_{on}$ and $\lambda_{off}$.

Atmospheric temperature profile measurement by DIAL technique

Simultaneously remote measurements of atmospheric temperature and humidity have been done using a tunable LIDAR. Good sensitivity for the humidity as well as the temperature measurements have been obtained [2]. For temperature measurements the DIAL technique is extended by using three wavelengths, two corresponding at the absorption of water vapor (0.72 µm or 1.9 µm) but originating from different ground state energy and the third wavelength is tuned away from the absorption line. The relation between the intensity of the lines and the temperature for two lines of differenting ground state energies $E_1$ and $E_2$ is given by:

$$\ln \left( \frac{I_1/I_0}{I_2/I_0} \right) = C + (E_2 - E_1)/kT \quad (10)$$

where $C$ is a function of the absorption cross section at the wavelengths $\lambda_1$ and $\lambda_2$. The sensitivity of the method is about 0.5 K.

Differences between a DIAL in the UV—visible and in the IR

The choice for IR or UV—visible is primarily determined by the existence of absorption bands (free from interferences with other gases) strong enough to permit a measurable difference of absorption in the optical path at the commonly occurring concentration values of the gas concerned.

Most of the absorption spectra of many gases lie in the IR range, whereas few of them are in the UV—visible at wavelengths free from absorption of the main air components. In Figs 4 and 5 are shown the trans-
mission of the atmosphere in the UV-visible and IR region, the absorption range of some pollutants and the available lasers.

From the eye-safety point of view the IR range (above 1.4 μm) and the UV-range (under 0.4 μm) present a higher damage light intensity level than the visible.

Yet in the IR there are the following disadvantages:
1) there are not laser sources that are together powerful and continuously tunable (as are dye laser in the visible UV);
2) infrared detectors (photodiodes and photoconductors) are much less sensitive than photomultipliers;
3) back-scattering coefficients are lower.

For these reasons, while in the UV-visible it is easy to obtain a range-resolved DIAL system, in the IR this would require very powerful lasers. Therefore most of IR DIAL systems need topographic reflectors. In the Table 1 are reported several gases detected by DIAL techniques both for UV-visible and IR systems.

Performances of DIAL system

The performances of a DIAL system depend of many parameters and the best method of optimizing is by means of a computer simulation. In any case the most important parameters which determine the performances of the system are the following:
- uncertainties in the differential back-scatter and transmission coefficients of aerosols;
- temporal variability of the LIDAR signal due to the natural fluctuations of the atmosphere particulate content;
- fluctuations of the laser emission wavelength;
- interference effect due to the absorption from other gases;
- meteorological visibility: there is an optimum meteorological visibility; for visibilities greater than the optimum value, the return signal is reduced because of less back-scatter, for visibilities less than the optimum, the return signal is reduced because of increased attenuation due to scattering. This optimum value of visibility depends on LIDAR range and on wavelength; typically for IR system the optimum visibility is about 1 km, in the UV is 20 km;
- detector noise: the signal to noise ratio is given by:

$$ s = \frac{P_T}{n} \frac{\Delta f + \left[ \frac{2 (\text{NEP})^2 \Delta f}{\eta} \right]^{1/2}}{\text{h} \nu} $$

where $P_T$ is the received power, NEP is the detector noise, $\eta$ is the detector quantum efficiency, $\Delta f$ its bandwidth, $h\nu$ the energy of the photon.

The first term in the denominator characterizes shot noise, the second dark current noise.

Using photomultiplier (UV-visible DIAL) the detection is limited by shot noise, in the case of IR detectors the dark current or background term is the most important.

The related concentration error is given by:

$$ \epsilon = \frac{n/s}{\Delta \sigma L m} $$

where $n/s$ is signal to noise ratio, $\Delta \sigma$ is the differential absorption cross-section at $\lambda$ on and $\lambda$ off m are the number of laser shots, and L is the spatial resolution.

Table 1. – DIAL applications

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Wavelength</th>
<th>Laser source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature and pressure</td>
<td>769 nm</td>
<td>Dye laser or alexandrite</td>
<td>10</td>
</tr>
<tr>
<td>Temperature and H₂O (Proposal)</td>
<td>720 nm</td>
<td>Dye laser or alexandrite</td>
<td>2</td>
</tr>
<tr>
<td>H₂O</td>
<td>720 nm</td>
<td>Nd-YAG + DYE laser</td>
<td>11</td>
</tr>
<tr>
<td>SO₂</td>
<td>300 nm</td>
<td>&quot;</td>
<td>11, 12, 13</td>
</tr>
<tr>
<td>O₃</td>
<td>300 nm</td>
<td>&quot;</td>
<td>11, 12, 13</td>
</tr>
<tr>
<td>NO₂</td>
<td>448 nm</td>
<td>&quot;</td>
<td>12</td>
</tr>
<tr>
<td>NO₃</td>
<td>480 nm</td>
<td>&quot;</td>
<td>13</td>
</tr>
<tr>
<td>Hg</td>
<td>250 nm</td>
<td>&quot;</td>
<td>13</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>10 μm</td>
<td>CO₂</td>
<td>14</td>
</tr>
<tr>
<td>H₂O, O₃, NH₃, SF₄, C₂H₄</td>
<td>10 μm</td>
<td>&quot;</td>
<td>15</td>
</tr>
<tr>
<td>Ethylene</td>
<td>10 μm</td>
<td>&quot;</td>
<td>16</td>
</tr>
<tr>
<td>CO₂</td>
<td>10 μm</td>
<td>&quot;</td>
<td>17</td>
</tr>
<tr>
<td>CH₄</td>
<td>1.6-3 μm</td>
<td>OPO</td>
<td>18</td>
</tr>
</tbody>
</table>
For $\text{SO}_2$, and our system with $L = 15$ meter, $m = 1000$, we obtain for the following values in ppb, as a function of range and visibility:

<table>
<thead>
<tr>
<th>Visibility</th>
<th>1 km</th>
<th>2 km</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 km</td>
<td>20 ppb</td>
<td>190 ppb</td>
</tr>
<tr>
<td>10 km</td>
<td>13 ppb</td>
<td>70 ppb</td>
</tr>
<tr>
<td>20 km</td>
<td>10 ppb</td>
<td>23 ppb</td>
</tr>
</tbody>
</table>

**LIDAR equipment**

In Fig. 6 is reported the block diagram of a typical DIAL system that consists of a laser source, a telescope which collects the back-scattered signal, a detector, a transient recorder for digitizing the LIDAR signal, an electronic system for timing and control connected to a minicomputer for data acquisition and automatic scanning.

**Laser sources.** – The development of the LIDAR is strongly dependent upon the evolution of laser technology. Nd–YAG laser is at the moment the best choice because it can provide high energy ($> 1$ Joule), high repetition rate (20 Hz), short pulse duration (10-20 ns) with high efficiency. Output frequency can be converted in several harmonics, and the frequency can be easily shifted in an efficient way by Raman technique. Moreover Nd–YAG lasers can generate tunable sources in UV–visible or in IR, pumping dye laser and optical parametric oscillator [3,4].

New, very interesting solid state laser, synthetic Alexandrite, is now commercially available. This laser is tunable in 700-815 nm range and could be very useful for LIDAR application [5].

Carbon dioxide laser also is widely used in LIDAR system, and high repetition rate, short duration pulses in very simple device have been realized [6]. Moreover it is easy to obtain narrow band operation with the possibility of utilizing heterodyne detection technique.

**Receiver system.** – The main component is a telescope of large aperture. It generally used a Newtonian or a Cassegrain configuration; the last is more compact. For a UV–visible system, a variable aperture is placed at the telescope focus and this defines the field of view. After this, a lens behind the aperture, produces a parallel beam that passes through the background blocking filters into the detector.

A photomultiplier is used for UV and visible regions with a quantum efficiency of about 20%. The linearity of response is extremely important and the peak anode current should be kept well below the maximum specified. Also dynamic range is important and generally gain modulation as a function of $R^2$ (or $P^2$) is used.

For IR DIAL liquid nitrogen cooled detectors are used; usually InSb up to 5.3 μm and HgCdTe up to 13 μm.

Detector noise is mainly due to the background thermal radiation seen by the detector; the noise can be reduced by changing the detector field of view. For wavelengths greater than 3 μm the intensity of skylight corresponds closely to that 300 K blackbody, so only cooled filters can reduce noise.

A 1 mm diameter detector seems to give the best compromise when used in a 2 meter focal length telescope.

**Digitizing of the signal.** – Two parameters of the digitizer are significant: sampling rate and number of bits. 10 bits, 10 MHz digitizers are currently available and utilized. When the on and off resonance wavelengths are produced by two separate lasers, these are normally fired about 100 μs apart and the two return signals are recorded in a single digitizer sweep.

**Computer control.** – Computer control is essential for any DIAL system.

The computer is utilized for: 1) laser tuning; 2) laser firing; 3) data acquisition and digitizer control; 4) taking data from energy monitors and the calibration system; 5) steering the telescope and the laser beam to produce 3D maps of pollutant concentrations; 6) data analysis. This involves converting the raw data from the digitizer into range resolved concentration maps.

**Description of two DIAL systems developed in CISE**

Two Differential Absorption Lidar (DIAL) systems have been developed at CISE laboratories, to measure atmospheric distribution and concentration of many gases [7]. The first is a mobile system with emission in the UV–visible range, committed by ENEL. Primarily intended for $\text{SO}_2$ [8] monitoring, its application can be extended to other gases ($\text{NO}_2$, $\text{H}_2\text{O}$, $\text{Cl}_2$, $\text{I}_2$, etc.) and other atmospheric parameters (temperature, pressure, aerosols).

The second, with emission in the IR (continuously tunable from 1.5 to 4 μm) has been developed for probing many pollutant gases: $\text{CO}$, $\text{CO}_2$, $\text{CH}_4$, hydrocarbons $\text{HCl}$, $\text{H}_2\text{S}$, $\text{NH}_3$, etc.

**UV–visible DIAL system**

This system utilizes a doubled dye laser pumped by the second harmonic of a Nd–YAG laser with 20 Hz of
frequency repetition rate. The emission at \( \lambda_{on} \) and \( \lambda_{off} \) is changed shot by shot by means of a device that inserts a prism in the dye laser oscillator cavity; in this way we deflect the laser beam on the grating of the laser cavity that selects the wavelength. The two wavelengths utilized for \( \text{SO}_2 \) measurements are shown in Fig. 7.

![SO_2 absorption spectrum](image)

**Fig. 7.** \( \text{SO}_2 \) absorption spectrum

The laser beam, by means of steering optics, is coaxially transmitted to the telescope. This can rotate over a solid angle of 2 \( \pi \) with a precision of 0.1° in zenith and 0.25° in azimuth.

The optical signal is detected by a photomultiplier, whose gain is modulated in time with a quadratic law. In this way the dynamics of the LIDAR signal is greatly reduced with improvement of precision in the analog-to-digital conversion. The digital signal is then transmitted to a Data General NOVA 4/S minicomputer.

Through the electronic control and timing system the minicomputer is able to make fully automatic measurements. It is possible to program telescope rotation, laser firing, wavelength change and other functions. The software permits measurements over a fan of directions, with real time data processing. The results, i.e., the concentration as a function of distance, are stored on a diskette and are subsequently recalled to get a concentration map using a plotter.

Table 2 summarizes the system specifications. The system is equipped with remote control of laser beam alignment. A TV camera is mounted on the telescope for eye safety and pointing control. The total time taken for the measurement in one direction, including telescope movement, is about 10°–30°.

The system is mounted on a truck and is equipped with a power generator of 50 kVA. The set-up time is about two hours.

<table>
<thead>
<tr>
<th>Transmitter</th>
<th>Receiver</th>
<th>Performances</th>
</tr>
</thead>
</table>
| Laser system: Nd-YAG pumped dye laser at 330 nm: 300 mJ (aerosol) at 300 nm: 10 mJ (SO₂) at 490 nm: 10 mJ (NOₓ) at 720 nm: 30 mJ (H₂O) Laser bandwidth: 0.1 cm⁻¹ Repetition rate: 20 Hz Pulse duration: 10 ns Telescope area: 0.25 m² Optics efficiency: 20% (SO₂) Field of view: 1 mrad Detector: Photomultiplier Philips XP-2020Q Detector specifications: 20% quantum efficiency Transient recorder: Biomation 1010 – 10 bit, 10 MHz Range: till 3 km Sensibility: 50 ppb (SO₂) Spatial resolution: 15 to 200 m Telescope angle resolution: 0.1° in zenith 0.25° in azimuth

In Fig. 8 we report a typical DIAL detection of a plume from a power plant with the two LIDAR signals. An example of a concentration map is shown in Fig. 9. This measurement has been made during a campaign in Southern Italy. The LIDAR was set between an industrial area and the centre of the town, at about 1.5 km from both.

![DIAL detection](image)

**Fig. 8.** Back-scattered signals and \( \text{SO}_2 \) concentration measurements
Fig. 9. — Map of SO₂ concentration obtained in a 2.5 km range and a 90 m spatial resolution.

Because of the good visibility, we used the system with a range of 2.5 km, so that it was possible to inspect all the areas of interest. The campaign for monitoring gaseous dispersion in different atmospheric conditions, had a duration of four days, for a total of 40 hours of measurements.

During this year the system has been tested in many campaigns in different experimental conditions as described in Table 3.

Table 3. — CISE—ENEL DIAL programs (up to June 1983).

<table>
<thead>
<tr>
<th>Type of measurements</th>
<th>N. of hours of work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power plant plume</td>
<td>80</td>
</tr>
<tr>
<td>model validation</td>
<td></td>
</tr>
<tr>
<td>Refinery</td>
<td>30</td>
</tr>
<tr>
<td>Urban area</td>
<td>30</td>
</tr>
<tr>
<td>VI Europea campaign of meteorological survey (FOS—Marseille)</td>
<td>80</td>
</tr>
</tbody>
</table>

IR DIAL system

The IR DIAL is similar to the UV-visible DIAL apart from laser system and detector. The laser consists of an Optical Parametric Oscillator (OPO) and Amplifier (OPA), pumped by a Nd—YAG laser (Fig. 10).

The characteristics of transmitter and receiver systems are reported in Table 4. The OPO is tunable in the range 1.5–4 μm with a bandwidth lower than 0.2 cm⁻¹. The wavelengths λ_on, λ_off are changed shot by tilting the etalon in the parametric cavity.

The etalon is mounted on a galvanometer, whose position is controlled by a minicomputer. The detectors are Indium Arsenide or Indium Antimonide, both cooled at 77 K, with field of view of 15° and detectivity better than 3.10¹¹ cm · Hz¹/₂ · W⁻¹.

The IR DIAL system shown in Fig. 10 was utilized for a preliminary campaign of measurements of CO concentration in a steel factory [9]. The absorption spectrum of CO measured with DIAL system is shown in Fig. 11. For field measurements the laser was tuned at 2.35 μm. Finally, in Table 5, we report proposal specifi-

<table>
<thead>
<tr>
<th>Table 4. — IR DIAL system characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmitter</strong></td>
</tr>
<tr>
<td>Laser system                             : YAG pumped parametric laser</td>
</tr>
<tr>
<td>Tunability                                : 1.5 – 4 μm</td>
</tr>
<tr>
<td>Energy output                             : 3 mJ at 2.35 μm</td>
</tr>
<tr>
<td>Laser bandwidth                          : 0.2 cm⁻¹</td>
</tr>
<tr>
<td>Repetition rate                           : 10 Hz</td>
</tr>
<tr>
<td>Pulse duration                            : 20 ns</td>
</tr>
<tr>
<td><strong>Receiver</strong></td>
</tr>
<tr>
<td>Telescope                                 : Area 0.2 m²</td>
</tr>
<tr>
<td>: Focal length 2 m</td>
</tr>
<tr>
<td>: Field of view 0.5 mrad</td>
</tr>
<tr>
<td>: Optical efficiency: 50%</td>
</tr>
<tr>
<td><strong>Detector</strong></td>
</tr>
<tr>
<td>InAs</td>
</tr>
<tr>
<td>Temperature 77 K                          : 77 K</td>
</tr>
<tr>
<td>Dimensions 1 x 1 mm                       : 1 x 1 mm</td>
</tr>
<tr>
<td>Detectors 3·10¹⁴ cm Hz ¹/₂ W⁻¹             : 5·10¹² cm Hz ¹/₂ W⁻¹</td>
</tr>
<tr>
<td>Rise time &lt; 0.1 μs                         : &lt; 0.1 μs</td>
</tr>
<tr>
<td>Field of view 15°                          : 15°</td>
</tr>
<tr>
<td>Transient recorder :</td>
</tr>
</tbody>
</table>
Conclusions

LIDAR techniques are now mature. A broad range of capabilities has been obtained. Routine measurements of cloud height, cloud cover, aerosol, smoke plume densities and concentrations measurements of gases are feasible. The main requirement is now to engineer safe and economical systems. It is important also to reduce the operating cost. Adequate technology is now available.

In other areas, like LIDAR temperature measurements, Doppler wind sensors, measurements of other pollutants, technological development is still needed. Moreover, LIDAR applications from a space platform offer unique capabilities to probe the atmosphere with high spatial resolution and to provide global observations of many atmospheric species and parameters.

Table 5. — Proposal laser system for the detection of CH₄

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Cise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser</td>
<td>Pulsed Nd–YAG</td>
</tr>
<tr>
<td>Wavelength</td>
<td>3 µm visible</td>
</tr>
<tr>
<td>Power</td>
<td>60 kW (peak power)</td>
</tr>
<tr>
<td>Energy</td>
<td>1 mJ</td>
</tr>
<tr>
<td>Pulse duration</td>
<td>15 ns</td>
</tr>
<tr>
<td>Repetition rate</td>
<td>up to 30 Hz</td>
</tr>
<tr>
<td>Laser cooling</td>
<td>air</td>
</tr>
<tr>
<td>Power requirement</td>
<td>1 kW</td>
</tr>
<tr>
<td>Weight</td>
<td>40 kg</td>
</tr>
<tr>
<td>Dimensions</td>
<td>(500 x 150 250) mm³</td>
</tr>
<tr>
<td>Temperature range</td>
<td>-10 °C to 40 °C</td>
</tr>
<tr>
<td>Humidity</td>
<td>95%</td>
</tr>
<tr>
<td>Vibrations</td>
<td>0.5 g up to 100 Hz 5 g</td>
</tr>
<tr>
<td></td>
<td>shock</td>
</tr>
<tr>
<td>Receiver area</td>
<td>200 cm²</td>
</tr>
<tr>
<td>Detector (with Peltier cooling system)</td>
<td>AsIn at 200 K</td>
</tr>
<tr>
<td>Response time</td>
<td>30 ms</td>
</tr>
<tr>
<td>Altitude range</td>
<td>0 to 500 m</td>
</tr>
<tr>
<td>Illuminated area (from 100 m)</td>
<td>0.6 m²</td>
</tr>
<tr>
<td>Measurement range (*)</td>
<td>200 ppm•m to 30,000 ppm•m</td>
</tr>
</tbody>
</table>

(*) Natural content of methane in air is 1.5 ppm. An 100 m overflight means to have a 300 ppm•m natural background.

Acknowledgements

The authors would like to acknowledge the research support provided by the Centro Ricerche Termiche Nucleari of the ENEL.

REFERENCES


MASS SPECTROMETRY IN THE FIELD OF CLINICAL BIOCHEMISTRY

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Summary. — Mass spectrometry technique offers at the present the best available method for the unequivocal identification of endogenous or exogenous substances from biological materials. Representative compounds measured in this way include biogenic amines and their precursors and metabolites, polyamines and drug metabolites. From the clinical standpoint the investigation of these substances contributes to confirm the diagnosis of pathological disorders, and adds to understanding of biotransformation, pharmacokinetic and drug metabolism. In clinical toxicology mass spectrometry is an essential tool in identifying substances in patients who are suffering from either accidental or professional exposure. Mass fragmentographic analysis permits the measurement of levels of many diagnostic compounds, such as uric acid, cholesterol, catecholamines, prostaglandins etc., with high accuracy, precision and sensitivity. Routine and reference methods may be calibrated against mass spectrometry which represents a definitive assay method.

Riassunto (La spettrometria di massa nel campo della biochimica clinica). — Allo stato attuale le applicazioni della spettrometria di massa ai problemi di analisi chimico-cliniche riguardano l’identificazione di prodotti endogeni o esogeni nei liquidi biologici (per es. catecolamine, pollamine, metaboliti di farmaci), la cui presenza serve per individuare particolari stati patologici, per seguire la trasformazione, la farmacocinetica e il metabolismo di farmaci, o per la identificazione di prodotti tossici assunti accidentalmente o professionalmente. Da un punto di vista quantitativo, la frammentografia di massa permette di determinare i livelli di prodotti aventi valore diagnostico (acido urico, colesterolo, catecolamine, prostaglandine, ecc.) con grande accuratezza, specificità e sensibilità, e costituisce un metodo di analisi definitivo rispetto al quale vengono calibrati i metodi di routine e di riferimento.

Introduction

By combining a gas chromatograph with a mass spectrometer (GC–MS) a system is formed by which the best of techniques for separation of complex analyte mixes (i.e. the gas chromatography) is employed with an excellent means of structure identification as is mass spectrometry.

As opposed to conventional gas chromatography, in this system the gas chromatograph uses the mass spectrometer as a highly specific detector in order to identify, without doubt and even in the presence of interferences, a single substance using the analytical information deduced from scanning and mass fragmentography (more precisely defined as the “Selected Multiple Ion Detection” — SMID) comparing to a standard.

At present the use of GC–MS in the field of clinical biochemistry is limited as instrumentation is highly sophisticated and costly while high running costs are entailed.

The aim of this study is to illustrate the use of this technique in the analysis of biological materials and, to better appreciate its potentiality, we have included a description of its principles.

Methodology and instrumentation

Principle of combined gas chromatograph—mass spectrometer

In this system the fundamental processes are the separation of the molecules of the sample, their ionization, the analysis of the ion masses produced and their monitoring. Separation of analytes is carried out with a gas chromatographic column in an oven and ionization produced by electron impact from a rhenium filament at a power of between 20 and 70 eV. The ions resulting from fragmentation of an analyte are then accelerated at ion accelerating voltage (several thousand of Volts) before passing through a narrow exit slit and electrode. Finally, the ions are conveyed into an analyzer tube in which, because of the continuously variable magnetic field produced by an electro–magnet, process called “scanning”, they are obliged to follow a trajectory curve at a speed proportional to their mass. After passing through a second slit these ions end up
colliding on an electron multiplier generating a signal proportional to the number of ions collected. The registration of these signals is called mass spectrometry on which, by means of an accessory called mass marker, identification of ion masses can be easily carried out. In this way a compound is identified by comparing retention time (deduced from the total ion current recording produced by the above-mentioned electrode), and the mass spectrum with a standard. Finally, monitoring of ions produced by fragmentation of the analyte under examination is carried out by mass fragmentography.

Mass fragmentography

When dealing with complex analytes, for example biological liquids, often the mass spectrum of the substance being examined is difficult to determine because of the presence of interfering elements which under gas chromatography behave as the substance itself. To overcome this difficulty a device was conceived which when incorporated in the mass spectrometer allowed only certain ions produced by the fragmentation of the examined substance to reach simultaneously the electron multiplier. In this way, a graph similar to a gas chromatogram, called “mass fragmentogram”, results from which information can be obtained for identification in the form of retention time and relative intensities of selected ion fragments which should be as the relative intensities of the ionic fragments in the mass spectrum of the compound in examination.

Fragmentography is also used when the substance to be examined is less than 10-100 ng which is the minimum level below which it is possible to obtain a mass spectrum. With fragmentography, in fact, even though poor in structural data, yield is improved 1,000-10,000 times and therefore it is possible to detect quantities of the order of picograms. Quantitative analysis is carried out by the internal standard method (isotope dilution principle) common to gas chromatography using however whenever possible as the internal standard the same substance under examination labelled with ²²Na or with ¹³C or ¹⁵N, minimizing errors due to the procedural losses.

Definitive assay methods

In practice the terms “routine method”, “reference method” and “definitive (or absolute) method”, even though still open for discussion, are normally intended as follows:
- routine: a method which does not permit errors to be detected during the individual phases of the analysis;
- reference: a method of measurement or analysis or test using reference materials of defined composition (internal standards or reference sera). It gives a slightly less accurate result than an absolute method;
- definitive: an entirely accurate method of analysis.

Even though reference materials are used the analytical results are highly accurate, i.e. free of systematic errors. In clinical biochemistry the necessity to be more or less accurate depends on the type of work to be executed. If the analysis is for scientific purposes extreme accuracy is required whereas a less level of accuracy can be justified in the case of routine analysis where procedure time is shortened as compared to more accurate methods or results are for diagnostic use. In both cases however it is important to evaluate the accuracy, that is to determine the difference between true value and analysis result; in practice this is achieved evaluating the difference between the results obtained with a reference method and those obtained by a routine method.

Isotope dilution–mass fragmentography, initially used in the field of pharmacology and only in part clinical biochemistry, has a relatively small level of inaccuracy and is proposed for the development of “definitive methods”. The high accuracy of this measuring technique is due to its high specificity (which is a characteristic peculiar to mass spectrometry) as compared to other existing reference methods, as well as precision in the recovery evaluation of a method using isotope dilution and the thorough examination it permits of possible errors in each single phase of analysis.

This high level of specificity is demonstrated in that, even if there are molecules which interfere with the fragmentographic analysis by producing ions which overlap those of the substance under examination, there is often the possibility to use other ions of a different mass to the interfering ions and which have an identical intensity ratio to those of the pure compound.

The possibility of having an exact recovery evaluation, and therefore produce extremely accurate results, comes from the fact that after having added the labelled molecules to a predetermined volume of serum or urine and after having subjected the sample to extraction or eventual chromatographic purification, the ratio between labelled and non-labelled molecules is maintained constant because of parallel behaviour during the various analytical phases.

Applications

The gas chromatography combined with mass spectrometry has proved, over the last few years, to be of considerable use particularly in the field of clinical biochemistry and medicine in general. Some examples of its application can be found in the study of the metabolism of drugs, in the field of clinical toxicology and in determining levels of compounds for diagnostic purposes.

Drug studies

In general, knowledge of a drug’s metabolism is useful for interpreting its pharmacological effect and can in many cases form a rational basis for therapy.
In fact, during the metabolic process, it is possible that substances more active than the drug itself are produced, sometimes even toxic, or the drug can be metabolized too quickly becoming inactive and thereby reducing in a brief space of time its pharmacological effect.

Apart from conventional methods, the initial identification of metabolites can be performed by administering the active principle and the same labelled with a stable isotope and recognizing the distinctive doublet pattern of ions in metabolite spectra. Frequently the structure of metabolites can be determined from their spectra and, once characterized, may be monitored by “selected ion” methods [1].

The dosage of drugs is important also in pharmacokinetics; however at this stage the use of mass spectrometry in this area is justified only in those cases where alternative methods are not sensitive or specific enough or other wise as confirmation of analytical results obtained by other methods.

Another area of development for mass spectrometry is the evaluation of drug concentration in biological fluids in relation to the pharmacological aspects. However, to present, data referring to the relationship between drug concentration and its therapeutic effect are rather limited even if there are however certain drugs for which it has been established the optimal range of therapeutic concentration in plasma (for example diphenylhydantoin, digoxin, phenylbutazone, and procainamide).

Clinical toxicology

The identification and dosage of toxic substances and their metabolites in human biological liquids is one of the principal interests of clinical toxicology as well as being of great importance in pharmacology.

Generally samples to be analyzed come from hospital patients who have been exposed either accidentally or intentionally to a toxic substance or have taken an overdose of one or more active therapeutic principles. It is obvious that to intervene with the right antidote first of all it is necessary to identify the compound which is the cause of the damage. This can be complicated when the compound has been swallowed in minute quantities, toxic nevertheless, or worse still when the damage produced derives not from the substance taken but from the new substances which it has generated, i.e. the metabolites.

Compared to the majority of conventional techniques, which are satisfactory only in the case of confirming the presence of these toxic compounds, mass fragmentography permits the identification and dosage of the various substances with security and precision even with small quantities (picograms). Moreover, today, the connection of a computer to the mass spectrometer gives in a shorter space of time a large quantity of data and makes this technique accessible also to operators not particularly specialized in mass spectra interpretation. The computer can in fact memorize the mass spectra of all the active therapeutic principles and their metabolites, of toxic substances in general and of the normal constituents in human biological liquids. In this way a continuous series of spectra can be produced during the elution time of a compound in the gas chromatographic column. Each spectrum is automatically compared to the other spectra in the computer memory to give all possible combinations and thereby producing the so-called “similarity index” of spectra indicating the degree of compatibility between them [2].

Prostaglandins

At the present, prostaglandins are the object of an in– depth study due to the fundamental role they play in human biochemistry as well as their potential therapeutic effect. Also in this case the combined gas chromatograph–mass spectrometer has been widely and efficiently used to identify the members of each of five classes of prostaglandins and the study of their biosynthesis and metabolism. The prostaglandins most commonly studied are the more active ones and belong to the series E and F [3–6].

Cholesterol

Cohen et al. [7] have developed a method, proposed as a “definitive method”, highly accurate and precise for the determination of total cholesterol in serum, adopting isotope dilution. Labelled cholesterol is added in a quantity nearly equal to the amount of total cholesterol present in serum, the concentration of which is known only approximately. After hydrolysis and extraction the cholesterol–$^2$H$_7$ and the cholesterol–$^2$H$_6$ are converted into trimethylsilyl ethers. With fragmentography, the intensity ratio between ions at m/z 465 (for labelled cholesterol) and at m/z 458 (for non–labelled cholesterol) is determined in accordance with a measuring protocol which includes the sequential measurement of calibration mixes made up of the trimethylsilyl ethers of the labelled and the non–labelled cholesterol, the ratio of which is very similar to that in the sample. The quantity of total cholesterol is determined on the basis of the amount of serum, the amount of cholesterol–$^2$H$_7$ added and the weight ratio of labelled to non–labelled cholesterol.

Uric acid

Öhmann [8] has developed a method for determining the dosage of uric acid in serum for use as a reference in evaluating the accuracy of various routine methods used in clinical biochemistry. An established quantity of uric acid, labelled with $^{15}$N, is added to serum and the mixture subjected to ion–exchange chromatography. After converting to tetra–trimethyl–
silyl ether, the mixture is analyzed with a multiple ion detection mass fragmentography using as diagnostic ions those at m/z 456 and 458.

This method can not yet be considered definitive in as much as the amount of error at each single phase of the procedure is not known. After a close examination of its inaccuracy, it will be possible to develop this method, which is however more specific and accurate than prior methods, into definitive method.

Cathecolamines

The identification and dosage with GC–MS in urine of certain important cathabolites of cathecolamines such as HVA, VMA, DOPAC and MHPG has proved to be very important in following the course of neuroblastom, an infantile tumor, in controlling the effect of treatment, discovering relapses and finally for prognosis. We have developed in our laboratory a method for identifying and determining dosage of HVA, VMA and MHPG [9] in urine using the corresponding deuterated compounds and a high-resolution capillary column for gas chromatographic separation.

Conclusions

The combined GC–MS at its present stage is the most versatile and accurate analytical technique available for determining volatile chemical species or substances made volatile by chemical derivatization in biological complex mixes.

It is considered indispensable in biochemical and clinical research.

The limited volatility of a chemical species, as such or derivatized and the absence in its mass spectrum of diagnostic fragments to use in fragmentography, are the only serious drawbacks for applying GC–MS to analytical problems.

The combination of a high pressure liquid chromatography to a mass spectrometer obviously overcomes the need for volatile substances. However, at this point there is the problem of interfacing which limits its adoption in laboratories.

The high cost of equipment, the necessity for specialized personnel as well as the running costs involved would convince the users to revert to this technique as an alternative only when there are problems of sensitivity and specificity in a particular analytical procedure or in the case of verifying the accuracy of a method.

In the field of clinical biochemistry today mass spectrometers are used for research and in centres specialized in studying metabolism for diagnostic purposes.

REFERENCES


STUDY OF THE DISTRIBUTION OF LEAD CONTENT IN BLOOD
BY MEANS OF ATOMIC ABSORPTION SPECTROMETRY

Z. NAGY, P. SERES, L. JÓNA, L. WINKLER and B. JUHÁSZ

Central Research Laboratory, Medicine School, University of Debrecen, Hungary

Summary. — The lead concentration of the blood of the adult population living in Debrecen and the surroundings was determined with the atomic absorption spectrometric method of Delves. The distribution of concentration and mean value were determined. The distribution was not normal, the mean value being 1.27 µmol/l (n=180). This value was in accordance with other values found in Hungary and western countries. The lead concentration of the mothers and infants' blood was also determined. Their mean values were nearly the same, i.e. 1.29 and 1.16 µmol/l, respectively.

Riassunto (Studio di distribuzione del contenuto del piombo nel sangue per mezzo della spettrometria di assorbimento atomico). — La concentrazione di piombo nel sangue della popolazione adulta abitante in Debrecen e dintorni è stata determinata per mezzo della spettrofotometria di assorbimento atomico secondo il metodo di Delves. Sono stati determinati la distribuzione ed il valore medio. La distribuzione non è risultata normale ed il valore medio è stato di 1.27 µmol/l (n=180). Questo valore è in accordo con gli altri valori trovati in Ungheria e nei paesi occidentali. E' stata anche determinata la concentrazione di piombo nel sangue materno e dei neonati. Il loro valore medio è quasi identico: 1.29 e 1.16 µmol/l, rispettivamente.

Introduction

Because of the accelerated industrial activity, civilization and motorizing our environment is becoming more and more contaminated with, among other elements, lead. From contaminated air, lead through food and beverages can infiltrate our organism. Some lipid soluble organic lead compounds can even penetrate the skin. From our intestines only 5-10% of lead is absorbed, while in the respiratory system the absorption rate is 30-50%. In 1961 the established maximal permissible dose was 0.6 mg/day and the "dose with zero effect" was 5-10 µg/day [1]. On the other hand, the daily uptake is estimated to be 30-150 µg.

In Hungary in the air–space of industrial workers the maximal permissible dose is 10 µg/m³ in an 8 h work period [2].

Lead is present in every mammalian organ; it is not an essential trace element, but it is absorbed from contaminated surroundings. It is a cell poison as it mostly inhibits, irreversibly, the enzyme reactions in the cell. It has been shown to block the active transport through the cell membrane by inhibiting the transport ATP–ases [3]. In the organism lead is accumulated in the bones, teeth and the aorta [4, 5]. The bone lead deposits remain unchanged for decades. Under the effect of lead in the bone marrow the red blood cell production is disturbed and abnormal, as can be seen in Fig. 1.

The young developing tissues of the organism and nervous system is especially sensitive to this metal [5]. The chronic damage caused by lead can be associated with kidney, nervous and vascular diseases.

![Figure 1](image-url) — The effect of lead on the biosynthesis of hemes.
The extended environmental contamination increases mankind's intake of lead. That is why it is important to determine the lead content in different biological materials.

Mobilized lead is mostly found in red blood cells (95%). The lead status of the organism, the limit of the storing capacity can be determined with the help of the blood lead content [1].

This is the reason why a specific and sensitive atomic absorption spectrophotometric microanalytical method was used in our experiments to determine the blood lead content ratio of the population in Debrecen and the surroundings.

The relation of this value to the safety tolerable levels was examined. We compared the lead concentration in a population which was exposed to great lead loading. It was interesting to know the concentration in mothers and infants who had an average lead exposure.

**Experimental**

**Material and method**

The blood received from the cubital vein of the population in Debrecen and the surroundings was examined. They differed in age (above 14), sex and occupation. Blood was received from the 1. Clinic of Surgery of the Debrecen University. Those who had high lead exposure were excluded. The navel–string blood of the infants and mothers came from the Gynaecological Clinic.

To our study an atomic absorption spectrophotometric lead microanalytical determination was used i.e. the modified method of Delves [6].

The former methods, unlike this one, were rather work and time-consuming (mineralization, extraction, etc.) and besides this several cm$^3$ s of the material were needed. Delves method differs from the other methods in the way of atomizing and the introduction of the sample. According to critical examinations [7], the method fulfilled all hopes, and compared to other AAS methods it gives similar results. It is advantageous because it is highly specific and from a small amount of sample (10$^{-5}$ cm$^3$) 1-10 nanogram lead can be determined. It is quite accurate, quick and suitable for screening examination. The sketch of the machine used to determine the blood lead content is seen in Fig. 2.

The vapour produced by the burning of the pretreated blood is directed from the small nickel cup into the quartz tube. The tube has side holes and is placed on a burner. A lead hollow cathode lamp produces the radiation which more or less penetrates this tube. The penetrated radiation intensity is detected after light resolution with a detector. The light intensity that reaches the detector parallelly decreases the amount of atomized lead in the quartz tube. The intensity was recorded as an absorption signal. During the whole absorpti

![Fig. 2. Scheme of instrumentation used to determine the blood lead content](image)

![Fig. 3. The absorbance signals recorded of original blood and after lead standard additions. The first high peak was not significant for lead caused by smoke, destroying the organic matter. This succeeds the small second peak (marked with X) which is significant for lead, and increased with the lead additions. Sometimes a third peak can be registered, not specific for Pb, caused by an unknown material](image)
to equal volumes of the original blood sample. The analytical signal of the original sample and of the samples obtained after addition were measured. From these the original blood lead concentration were calculated either graphically or with computer methods. In the graphical method the analytical signal is rendered to the standard concentration of blood samples. To the points defined by the additional concentrations and the corresponding analytical signal a direct line is fitted. The absolute value of the point of intersection with the horizontal axis – the negative concentration that gives zero absorbance – is characteristic to the original lead concentration. The principle of the graphical evaluation is seen in Fig. 4. Because of the large number of data we chose the computer method which was based on the numerical version of the above mentioned method. To

\[20 \mu l H_2O_2 (30\% w/v)\] was added to all cups. Then it was dried again. The cups were placed in the flame with a wire of resistance soldered in a glass stick. The cups were directed under the hole of the quartz tube. The distance between the nickel cup and the quartz tube was 1-2 mm that resulted in an optimal sensitivity and separation of signals. The recorded signals are seen in Fig. 3. The permanent place of the cup was ensured with a tripod.

**Instrumentation**

Atomic absorption spectrophotometer: Varian Techtron Model Type 1000. Wavelength: 283.3 nm, lamp current: 6 mA, slit: 0.2 nm, acetylene: 2.5 l/min, air: 5.5 l/min, system: transmission. Registration: recorder with a single channel: Radelkis OII 814 on a log paper for extinction registration (200 mm width). The paper velocity was 150 cm/h.

A tube adapter: was used instead of the nickel tube originally used by Delves [6], a quartz tube: inside diameter 13 mm, tube length 110 mm, side hole diameter 10-12 mm. The quartz tube had to be changed after 2-3 hundred measurements.

Sample holding cup: made of nickel foil (0.15 mm) by pressure. Inside diameter: 7 mm, height: 3.5 mm. After about 40-50 determinations new cups were needed.

**Results and discussion**

The relative frequency distribution of blood lead concentration of the adult population in Debrecen and the surroundings is shown in Fig. 5.

The mean value is 1.27 \( \mu \text{mol/l} \). The range is between 0.14 \( \mu \text{mol/l} \) and 3.2 \( \mu \text{mol/l} \). The values between the 10 and 90 percentile limits are 0.64 and 2.3 \( \mu \text{mol/l} \).

**Preparation of samples**

Twelve samples 4 times, 3 parallely, were measured. 40 \( \mu l \) alcohol, then 10-10 \( \mu l \) heparinized blood were added to the cups. Lead solution was not added to the first three samples. To the others an increased concentration of standard lead solutions 1.2 \( \mu \text{mol/l} \), 2.4 \( \mu \text{mol/l} \) and 4.8 \( \mu \text{mol/l} \) were pipetted, respectively. An Eppendorf automatic pipette was used. After drying,
The median is 1.1 \( \mu \text{mol/l} \). It is shown in the figure also that the concentration distribution of lead blood in the examined population has a certain tendency and does not follow normal distribution. This may be explained according to a certain author [21], that the lead does not belong to elements of homeostatic regulation. In our age the limit of safety in blood is: 2.9-3.8 \( \mu \text{mol/l} \). Above this limit, there is the suspicion of poisoning [1] and a therapy is necessary [11]. Compared to this value, we did not find pathological values in the examined population. In Table 1 our results were compared with those reported in the literature.

As can be seen, the results are in agreement on the whole. This means, that the extent of our lead exposition unfortunately reached the level of the population of developed countries. The cumulative frequency distribution of lead content of newborns blood and of the mothers is shown in Fig. 6.

The two mean values do not differ significantly. The average of maternal samples is 1.29 \( \mu \text{mol/l} \) and the newborns is nearly the same: 1.16 \( \mu \text{mol/l} \). Thus the infants have already as high a lead concentration level as do adults.

It was formerly established in human and animal experiments that lead penetrates the placenta [13, 14]. Thieme [15] determined the placenta lead concentration by a neutron activation method. Table 2 shows some lead concentrations in blood published recently [16-20]. In comparison, the concentration area found by us was in good correlation with literary data but our mean value was a bit higher.

![Cumulative frequency distribution of lead blood concentration of mothers and newborns](image)

Table 1. - Literature values reported for lead in blood of adults (\( \mu \text{mol/l} \))

<table>
<thead>
<tr>
<th>References</th>
<th>Study</th>
<th>Year</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Tompsett and Anderson (GB)</td>
<td>1935</td>
<td>2.6</td>
</tr>
<tr>
<td>5</td>
<td>Knoch et al. (USA)</td>
<td>1940</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>Knoch et al. (Mexico)</td>
<td>1940</td>
<td>1.3</td>
</tr>
<tr>
<td>1</td>
<td>Hardy et al. (USA)</td>
<td>1971</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>Hauser et al. (USA)</td>
<td>1972</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>Marcus et al. (USA)</td>
<td>1973</td>
<td>1.3</td>
</tr>
<tr>
<td>2, 12</td>
<td>Timár (II)</td>
<td>1961</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>This report (II)</td>
<td>1976</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 2. - Literature values reported for lead in blood of newborns (\( \mu \text{mol/l} \))

<table>
<thead>
<tr>
<th>References</th>
<th>Study</th>
<th>Year</th>
<th>Blood</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
<th>Method of analysis</th>
</tr>
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<tbody>
<tr>
<td>16</td>
<td>Kubasik and Volosin</td>
<td>1972</td>
<td>Capill.</td>
<td>20</td>
<td>0.7</td>
<td>0.3–1.1</td>
<td>AAS – CRA (*)</td>
</tr>
<tr>
<td>17</td>
<td>Robinson et al.</td>
<td>1958</td>
<td>Cord</td>
<td>10</td>
<td>0.8</td>
<td>0.3–1.3</td>
<td>Dithizone</td>
</tr>
<tr>
<td>18</td>
<td>Scanlon (**)</td>
<td>1971</td>
<td>Cord</td>
<td>28</td>
<td>1.0</td>
<td>0.5–1.9</td>
<td>&quot;</td>
</tr>
<tr>
<td>19</td>
<td>Harris and Holley</td>
<td>1972</td>
<td>Cord</td>
<td>24</td>
<td>0.6</td>
<td>0.5–1.0</td>
<td>&quot;</td>
</tr>
<tr>
<td>20</td>
<td>Rajegowa et al.</td>
<td>1972</td>
<td>Cord</td>
<td>100</td>
<td>0.7</td>
<td>0.5–1.4</td>
<td>AAS</td>
</tr>
</tbody>
</table>

AAS: Atomic Absorption Spectrophotometry
CRA: Carbon Rod Atomizer
**The data of Scanlon is the average of urban and suburban infants

REFERENCES


