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A single bacterium suffices to identify the nature of a disease

Diagnosis: meningitis. But what is the cause of this particular case? New techniques for analyzing cerebrospinal fluid, developed and tested at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw, make it possible to diagnose the bacterial cause of the disease in less than a quarter of an hour and to establish the species of the intruder on the basis of just a single bacterial cell.

Various factors can be responsible for causing inflammation of the meninges. The sooner the doctor knows the cause of a particular case, the sooner he can administer the right treatment and prevent the disease from reaching its critical stage, frequently resulting in coma or the patient's death. The existing methods of analysis do not make the task any easier – they require, for example, attempts to multiply bacteria, which could last even several hours. However, it turns out that the analysis time can be reduced to less than a quarter of an hour! Fast and reliable diagnosis of bacterial infections is now a reality thanks to new techniques for examining cerebrospinal fluid, developed at the Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS) in Warsaw. The test itself merely requires a microlitre sample of fluid, in which a single bacterium must be found in order to identify the species responsible for the disease.

The work on disseminating a highly sensitive analytical technique, SERS (Surface Enhanced Raman Spectroscopy), has been well under way at the IPC PAS . The main physical mechanism underlying this approach is the inelastic scattering of photons, known for dozens of years.

Typically, when a molecule absorbs a photon, it immediately emits another of the same energy – such scattering is defined as elastic. Sometimes, however, some of the energy of the absorbed photon is transferred to elicit vibration or rotation of the molecule and then the emitted photon has a little less energy. The opposite situation may also arise. The emitted photon has a little more energy, because the molecule transfers excess rotational or oscillating energy. Inelastic scattering is a rare phenomenon, it occurs in only one out of several million photons. Therefore, in normal conditions, Raman scattering signals are very difficult to record.

“The situation changes dramatically when the molecule is placed on an appropriately roughened surface. The Raman scattering signal is then amplified from a million to a billion times, and it can be observed without any major problems. This effect has been known since the mid-1970s,” explains Dr. Agnieszka Kaminska (IPC PAS).

In cooperation with the Institute of Physics of the Polish Academy of Sciences in Warsaw, the IPC PAS has developed a special substrate of zinc oxide which works like SERS-active platform. These substrates were used for SERS measurements of neopterin concentration in cerebrospinal fluid samples, provided by the National Medicines Institute (NMI) in Warsaw, from patients previously diagnosed with meningitis. The detected levels of neopterin turned out to be ten times higher than in a reference sample, taken from a healthy person.

An elevated level of neopterin is valuable information that the body is fighting a disease with bacterial aetiology. However, meningitis may be the result of infection caused by various bacterial species. To work really effectively, the physician should know which species he is dealing with.

The measurement of Raman spectra of bacteria in cerebrospinal fluid proved difficult. In contrast to chemical molecules that do not move on the substrate, bacteria are in constant motion. The scientists were thus faced with a challenge – to develop a substrate which would not only provide amplification of the Raman signal, but also filter out bacteria from the fluid and effectively immobilize them for the duration of the measurement. The solution turned out to be cheap, commercially available woven mats, covered at the IPC PAS with a thin layer of an alloy of gold and silver (its thickness is approx. 70-80 nanometers). A stream of cerebrospinal fluid administered via a syringe pump was then passed through a system of these mats, arranged with decreasing mesh sizes. When a bacterium reached mats with too small pores, it got stuck in one of them, and an appropriately selected flow rate of the fluid stream prevented it from changing its location.

“We have tested our substrates on three species of bacteria causing meningitis: N*eisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. We correctly detected their presence in 95% of cases and the species was identified with a certainty of up to 98%. And since we are talking about extremely sensitive Raman spectroscopy, the only requirement we had to meet in order to gain such precise results was to find one bacterial cell”, emphasizes Dr. Kaminska.

The entire course of analysis is largely automated and minimizes the lab worker's contact with the test sample. In order to perform the measurement it is merely necessary to draw a microlitre aliquot of the cerebrospinal fluid by laminar flow into a syringe, then place it in a syringe pump connected to the Raman spectrometer. In order to enhance the certainty of measurement, the recorded signals are processed by software that uses advanced statistical methods and the operator is finally obliged to read the result.

When compared to the existing methods, the solution proposed by the IPC PAS has a number of advantages: it requires only small amounts of cerebrospinal fluid and eliminates the need for long-term proliferation of bacteria. Furthermore, the automation of measurement ensures a high level of safety, and the result is available within minutes. An important argument is also the price: the purchase of equipment necessary to carry out the analysis does not exceed several thousand dollars, therefore even small medical clinics can afford it.

The Institute of Physical Chemistry of the Polish Academy of Sciences (http://www.ichf.edu.pl/) was established in 1955 as one of the first chemical institutes of the PAS. The Institute's scientific profile is strongly related to the newest global trends in the development of physical chemistry and chemical physics. Scientific research is conducted in nine scientific departments. CHEMIPAN R&D Laboratories, operating as part of the Institute, implement, produce and commercialise specialist chemicals to be used, in particular, in agriculture and pharmaceutical industry. The Institute publishes approximately 200 original research papers annually.

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SCIENTIFIC PAPERS:

"Rapid detection and identification of bacterial meningitis pathogens in ex vivo clinical samples by SERS method and principal component analysis"
A. Kamińska, E. Witkowska, A. Kowalska, A. Skoczyńska, P. Ronkiewicz, T. Szymborski, J. Waluk
Analytical Methods, Issue 22, 2016; DOI: 10.1039/c6ay01018k

LINKS:

http://www.ichf.edu.pl/
The website of the Institute of Physical Chemistry of the Polish Academy of Sciences.

http://www.ichf.edu.pl/press/
Press releases of the Institute of Physical Chemistry of the Polish Academy of Sciences.

IMAGES:

IChF160921b_fot01s.jpg **HR:** http://ichf.edu.pl/press/2016/09/IChF160921b_fot01.jpg
A single bacterium suffices to identify the species of the intruder. Fast and reliable diagnosis of bacterial infections is now a reality thanks to new techniques for examining cerebrospinal fluid, developed at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw. Pictured above: Dr. Agnieszka Kaminska in the lab. (Source: IPC PAS, Grzegorz Krzyzewski)