There’s anthrax in the air

Particle analyser recognises dangerous bacteria in an instant
Dr. Ir. Jan Marijnissen, particle technologist with TU Delft’s Chemical Technology section, has been busy developing a particle sensor for fourteen years now. Suddenly his work has become more topical than ever in the wake of the terrorist attacks in the States. A chemical particle sensor has meanwhile been launched on the market, and now the chemical technologist is working with doctorate student Arjan van Wuyckhuyse to develop a sensor that can trace bacteria and especially the seemingly ubiquitous anthrax bacteria. Hospitals, of course, will naturally find this method very useful, but it is the military who are showing particular interest.

On 19 March 1995 at 8:17 a.m., the Aum Shinrikyo sect (Doomsday Cult adherents) led by guru Shoko Asahara carried out an attack in the Tokyo subway system using the poison gas Sarin. The terrorist operation killed 11 people and injured 5,500.

Dr. Marijnissen studied mining engineering and specialized in the ventilation of (underground) mines. Aerosol particles floating around in the mine’s atmosphere had his special attention. After years of globetrotting, many of which were spent in Africa and the USA, some fifteen years ago he was asked by professor of chemical technology Brian Scarlett to come and set up an aerosol laboratory at Delft University of Technology. He was given five years to make it work, and another five years to put it among the world’s top-ranking laboratories. Looking back, he reluctantly admits that they made the grade. Like any good scientist, Marijnissen started by trawling through the literature. It seemed that the greatest need was for a chemical method that could be used to directly determine the chemical composition of airborne particles.

‘At the time, some fourteen years ago, several institutes were doing research on the subject. The research could be divided into two main streams. The first was a system in which the air samples were taken to a laboratory to be analysed using a microscope, laser, and mass spectrometer. The second, which was being worked on by, among others, Sheldon Friedlander in the USA, determined the aerosols directly using first an incandescent filament, and later a laser. However, this system used a mass spectrometer that showed only a limited part of the spectrum.’

What Marijnissen did, was simply combine the two to create a new method that eliminates the drawbacks. Friedlander’s filament was a bit of a problem, as it tended to break up large and fragile molecules and had difficulty vaporizing refractory materials, while indirect measurement was a shortcoming of the other system. Instead of a filament a laser was used, and the proposed mass spectrometer was the straight variant, a so-called Time-of-Flight model, unlike the models that make use of a varying electrical field to scan the mass range.
Marijnissen: ‘I presented my idea in 1988 at a conference on aerosol technology at Lund in Sweden. At the time, it was quite a hit, even though it was no more than an idea that I had cobbled together based on existing research work, with the added notion of using a straight mass spectrometer.’

Glass In the end, the idea simply had to be tried. The first system was made by TU Delft’s own glassworks. The mass spectrometer came from the Amolf Institute in Amsterdam and used what is known as a pulsed YAG laser. YAG indicates the crystals which determine the specific wavelength of the light beam. In Friedlander’s design, the filament performs the same function as the laser, vaporizing and ionising particles. Olaf Kievit was the first to work on the set-up as a student and later as a graduate. He was awarded his doctorate in 1995 for his thesis on the mass spectrometer. Although particle technologists are rather excited by Marijnissen’s analyser – new research groups were soon formed in the USA and in Germany – the analyser set-up didn’t really amount to much.

Marijnissen: ‘We did manage to get some spectra from it, but we didn’t have a clue what they meant. We published the results, but after that we started on the hard work, which was to design a working system.’

The resulting device was made in Delft from stainless steel. It consisted of three chambers in which the pressure was lowered in stages down to 10^-6 millibar (only slightly less than one billionth of one atmosphere). The near vacuum ensures that the particles are not hindered in their flight by air molecules.

The YAG laser used to «blast» the particles was assisted later on by a helium-neon laser. Prior to this, the YAG laser had been firing light pulses blindly at the particles entering the vacuum chamber. As soon as the helium/neon laser detects an incoming particle, the YAG laser is activated to perform its splitting act. The ensemble was completed by a straight spectrometer, a Time-of-Flight mass spectrometer. Olaf Kievit’s research prepared the ground for the final version of the particle analyser. He built and calibrated the system, so the spectra being produced by the mass spectrometer could actually be interpreted.

Sea air Although Kievit’s pioneering work looked promising, Marijnissen and his staff were still far from satisfied. Chemical engineer Martin Weiss was recruited to improve the particle analyser on a number of points, such as determining the particle size and the moment at which the big laser was to be activated. He also had the job of creating a library of spectra to simplify the identification of the spectra being measured (i.e. which peak represents which substance). Weiss analysed, among other things, the air in and around the Delft chemistry laboratory.

Marijnissen: ‘The strange thing was that sometimes we would see high sodium peaks, and at other times not. In the end, it turned out to be wind-related. With the wind coming from the west, it carried with it saline sea air. Winds from the east resulted in high calcium peaks. We think these are related to building activities.’
Presence detection by the helium/neon laser also was far from ideal. Although the little laser managed to spot the particle, it didn’t know what size it was, in other words, how fast or slow. As a result, the yag laser, after being given the «fire» command by the scattered-light sensor, was rather prone to missing the target. Weiss came up with the solution of splitting the helium/neon light beam into two parallel beams. Once a particle crosses both beams, its speed and size can be measured, and the yag laser stirred into action at the correct moment. Eureka!

‘People were also working on similar systems in other places, not only in the USA, where quite a bit of funding went into this kind of research, but also in Germany. We just carried on, and now the system is really working the way we want it to.’

Pudding Even so, the proof of the pudding is in the eating. The opportunity soon arrived.

Marijnissen: ‘Around that time, it must have been in ’95 or ’96, the Germans arrested a Russian who was carrying plutonium in the boot of his car with the intention of selling it. The Institute for Transuranic Elements at Karlsruhe wanted to know if our particle analyser could be used to foil similar feather-brained schemes. If accidents with nuclear reactors occur, information is quickly needed about any radioactive components that have entered the atmosphere. After the Chernobyl incident, for instance, it was quite some time before the radioactive isotopes that had escaped into the atmosphere were identified.

‘We devised a test rig, in which small ion exchanger beads were loaded with a thin layer of uranium oxide. Less than one four-hundredth of the uranium consisted of the radio isotope, U-235.’ Marijnissen shows the resulting spectra.

‘See, this is a peak produced by the radioactive U-238, and this peak represents the U-235. It shows up quite clearly.’

According to Marijnissen, ‘Karlsruhe’ was satisfied with the test results. Then the Institute asked if the particle analyser could also sniff out cannabis. However, this request hit a snag when Martin Weiss, who does not smoke, refused to light up a reefer. Actually smoking the stuff wasn’t necessary for the tests however, and once again the analyser performed without a hitch. The peak for the (tetrahydrocannabinol, the active compound of cannabis) stands out a mile from the measured spectrum. Hardly remarkable, since you don’t really need a sniffer dog to smell a joint from miles away.

Marijnissen: ‘We haven’t done any tests yet on people just in possession of cannabis.’

Soot Marijnissen and his team did carry out tests with soot, however.

‘Some researchers claim that soot particles are a major cause of death. In the U.S. alone, 30,000 people are thought to die each year as a result of soot. Nowadays diesel fuel contains a catalyst, an additive that is supposed to decrease the soot emission. We conducted the test on diesel soot with a cerium catalyst. The
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cerium showed up nicely.
We also conducted a test on anthracene, a polyaromatic hydrocarbon, that is found in soot. We could clearly identify the mass of the polyaromatic hydrocarbon that was adsorbed on the Norit we used to simulate soot.' The analyser could well prove useful for process control applications as well, or for monitoring the working atmosphere. In the pharmaceutical industry, it is essential to know whether substances are pure or not. It can also be important to know that the level of certain airborne bioactive substances, such as enzymes of hormones, is below acceptable limits.

Marijnissen: 'I had prepared a report of our findings for a British pharmaceutical company. One group of analysts was wildly enthusiastic, but another group was vehemently opposed. I suspect they were afraid of losing their jobs, but this type of analyser does not render all other analysis methods obsolete. For example, a mass spectrometer, cannot be used to distinguish one stereoisomer from another, i.e. to tell the left-hand form from the right-hand form, as it were. Being able to make that distinction is particularly crucial in the pharmaceutical industry. At the time when Marijnissen was applying for a patent on the Delft particle analyser, he had dealings with an American company about his research. Later on that company was bought by the tsi Inc. Two years ago tsi Inc. started to market a commercial system the size of an office cabinet. Marijnissen says that most of the device is based on the first generation of Delft particle analysers.

Bacteria By then, work was under way on a second generation of analysers

‘The equipment produced by tsi works really well,’ Marijnissen says, ‘but it has its limitations. The scattered-light detector cannot «see» particles smaller than 0.3 mm (3 times 10-7 m; AS). This is partly due to the wavelength of the laser light it uses. Moreover, the system can only be used to detect relatively small molecules with a molar mass less than 800 (the molar mass indicates how many times heavier a molecule is than a twelfth of a carbon atom, providing a measure for the size of the molecule; AS). This means that our system was unable to identify biological compounds. Given the risk of biological weapons being used by terrorist groups or rogue countries, no time was to be wasted in developing a method that could directly determine the nature of biologically active compounds or micro organisms.'

Threat Although the threat of biological weapons is not new, recent events in the United States have made it much more immediate. People in America would be willing to pay a lot for a bacteria sensor system which could detect the difference between the notorious anthrax powder and just plain flour which some jokers are sending through the postal services. The anthrax letters have already killed several innocent people. Three years ago, the tno Prins Maurits Laboratory approached Marijnissen. The result was a joint follow-up research effort on the part of doctorate student Arjan van Wuyckhuyse and research fellow Michael Stowers.
‘The small particles weren’t the real problem,’ Marijnissen says, ‘we simply make them grow by condensing auxiliary compounds onto them. This is a method we have mastered to perfection.’

Increasing the mass was a trickier business. The researchers had to prevent the larger, but often vulnerable, biological molecules from being blasted to smithereens by the laser. By then, the yag laser had been replaced by what is known as an excimer laser. There is a trick used in mass spectrometry called maldi (Matrix Assisted Laser Desorption Ionisation). Put less scientifically, the method consists of adding another material that absorbs the laser energy and promotes ionisation. As a result, the compounds that are the object of the exercise reach the detector unscathed, or most of them, anyway. There is another trick involved here, but Marijnissen is loath to talk about it because of a pending patent application. The new bio detection system includes a fluorescence detector that provides an indication of the presence of biological material. That’s all he’s willing to say for the moment. Apparently, during the Gulf War, U.S. troops, fearing that Saddam Hussein might resort to biological warfare, carried fluorescence meters, but they gave so many false alarms that in the end they were of little use. Research fellow Michael Stowers is working to improve the fluorescence section.

Conifers After his historical exposé, Marijnissen directs us to doctorate student Arjan van Wuyckhuyse, now in his fourth year of research, for details on the biological side. Van Wuyckhuyse shows a few spectra and points out some details: ‘This is where we measured insulin. We used coniferic acid, also known as ferulic acid, as the maldi compound. At first we used simpler compounds, such as nitrobenzyl alcohol, but compounds like coniferic acid yield better results with higher molar masses. Here you can see the peaks of the dimers and trimers, molecules consisting of two and three insulin molecules, respectively.’ He also shows spectra that reveal the presence of cytochrome-C and myoglobin. These are complex biological compounds. Myoglobin also occurs in bacteria, and in whale sperm for that matter, to mention a curious if not entirely relevant fact. Cytochrome-C, which does not occur in bacteria, has a molar mass of about 12,200; the molar mass of myoglobin is approximately 18,000.

In bacteria, you need to be able to measure compounds up to a molar mass of 40,000 in order to be able to identify them,’ Van Wuyckhuyse explains. In the United States, researchers at the University of Indiana have succeeded in using mass spectrometry analysis to identify 25 different types of bacteria. The process involved pretreated deposits on a sample plate being analysed by a maldi mass spectrometer. Van Wuyckhuyse: ‘The tests were successful, even after blind testing. Our goal is to be able to detect the proteins in bacteria.’

Anthrax In addition to bacteria, the Delft particle analyser can detect spores, which are rather like
hibernating bacteria.

‘We have conducted tests on spores of Bacillus subtilus var. niger’, Van Wuyckhuyse says, ‘which is a harmless relative of anthrax. The results showed a peak around molar mass 1220 which we couldn’t place. At that point they took another look at the sample using other equipment. This showed that the peak was caused by skeletal remains of B. subtilus. The peak is typical for that type of microbe. Several peaks together can result in a method for identifying spores and bacteria. However, it is going to take time to detect all these peaks for a single type of microbe.’

Although biological weapons have seen little action so far, Van Wuyckhuyse thinks their danger cannot be ignored. Some time after the interview his words prove prophetic as the States is overcome by a wave of anthrax cases. But it has happened before.

‘A few years ago, the Aum Shinrikyo sect released the chemical poison Sarin into the Tokyo subway system, causing many deaths and injuries. And a recent book by Russian defector Alibekov (who was the second man of the Soviet biological warfare programme) reveals that the same terrorists had tried before using the deadly anthrax. Fortunately they had taken the wrong strain, which was harmless. Our system could work well for these kinds of applications, but it would also work well in hospitals and sorting offices of postal services. Hospitals are going to have to wait a while longer. The instant bacteria detector isn’t ready yet, but it is only a question of time. N

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