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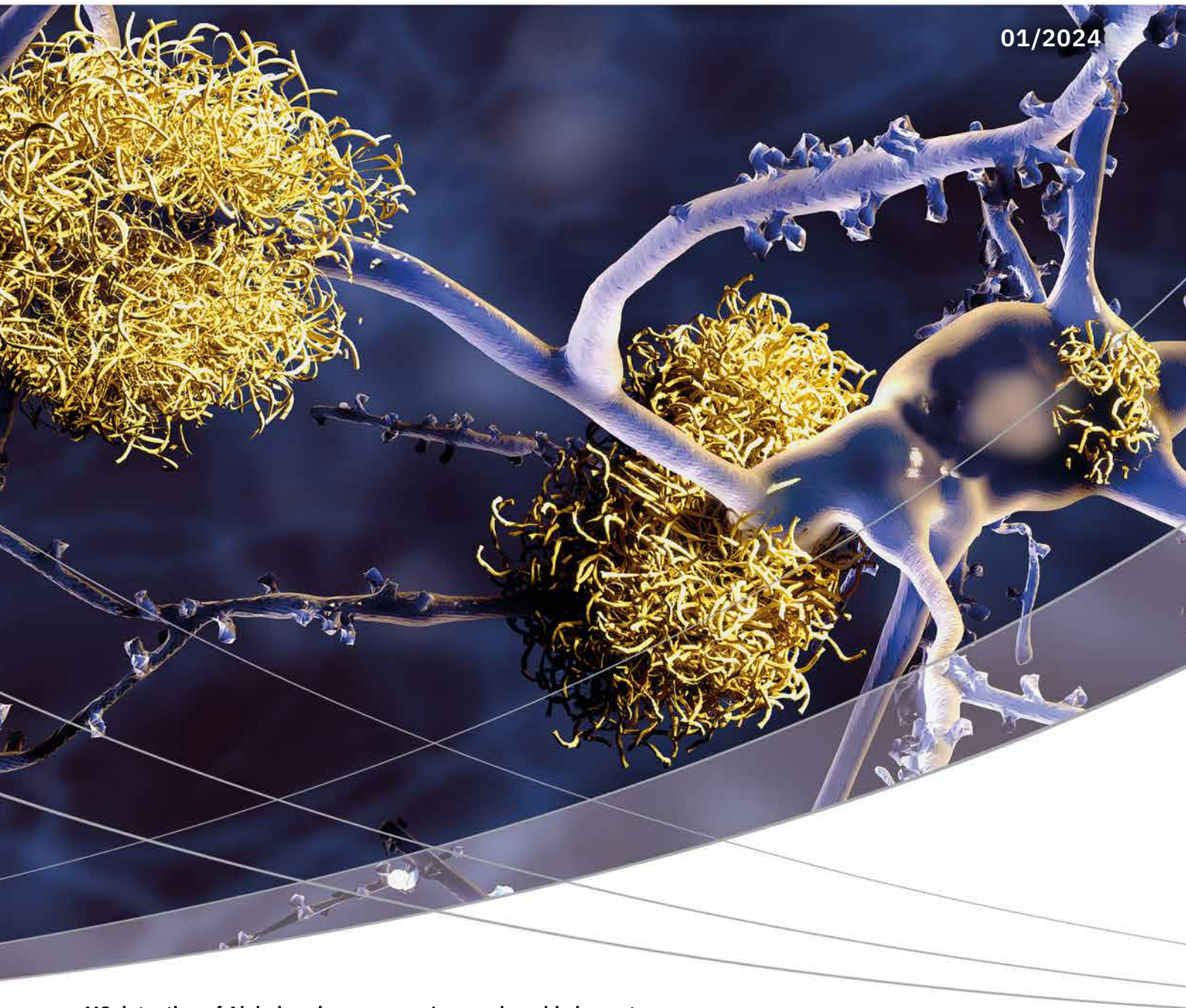

01/2024

MS detection of Alzheimer's blood-based biomarkers

From a few drops of blood – the first successful detection of plasma Aβ by MS

Low carbon, big impact: making the most of biowaste

Making useful materials from biological waste streams using pyrolysis

New trump cards for blood analysis

DPS cards – a new method of collecting and analyzing blood

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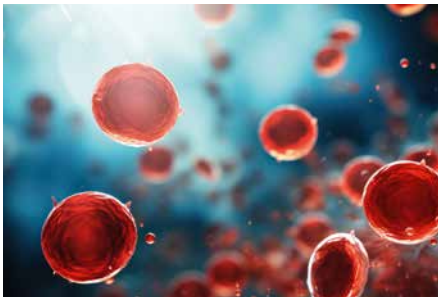

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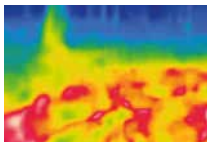

Hot on the heels of infrared radiation

A novel experiment for measuring thermal radiation

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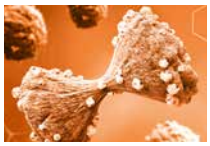

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Events

Hot on the heels of infrared radiation

A novel experiment for measuring thermal radiation

Dr. Benjamin Thomas, Shimadzu Europa GmbH

Heat transfer through infrared radiation occurs often in everyday life. Infrared heaters provide warmth in the living room, terrarium or canteen display. This type of heat transfer between particles also plays an important role in industrial processes, for example in iron processing or lime burning, where bulk material is heated in massive ovens.

One area of research at the department of Energy Plant Technology at Ruhr University Bochum, led by Martin Schiemann, is the investigation of this heat transfer between particles in packed beds. An arrangement of metal or mineral rods serves as a model system. A unique experiment based on a modified IRTracer-100 was developed for measuring the emissions.

Heat transfer from a warmer body to a colder one can basically take place in three different ways, as shown (Figure 1) in the example of a pot of water:

(A) Via convection with mass transfer in a mobile medium
(B) Via thermal conduction through a medium without mass transfer
(C) Via thermal radiation, which is also possible in a vacuum

In convection (A), differences in temperature are followed by differences in density, which cause molecules to move from high-pressure zones (warmer) to low-pressure zones (colder). In this experiment, for example, hot water rises to the top of the pot. Temperatures in both layers equalize when the two kinds of water are mixed.

In thermal conduction (B), heat is transferred by vibrations (non-metallic solids), impacts (fluids) or mobile electrons (metals), depending on the nature of the medium. The "warmer" molecule transfers energy to the "colder" molecule here.

This article takes a closer look at thermal radiation (C), which is where a hot body emits broadband radiation in the infrared spectral range. When a material absorbs this infrared radiation, this stimulates vibrations and rotations there again, which in turn heats the absorbing material.

Thermal radiation is the only type of heat transfer that does not require a medium. However, the heat is only transferred if the material actually absorbs this infrared radiation and does not reflect it. For this reason, an evacuated chamber with mirrored surfaces is used for insulation, e.g. in thermos flasks, to prevent all types of heat transfer. Heat lamps, on the other hand, specifically generate infrared radiation to transfer heat. ⟶

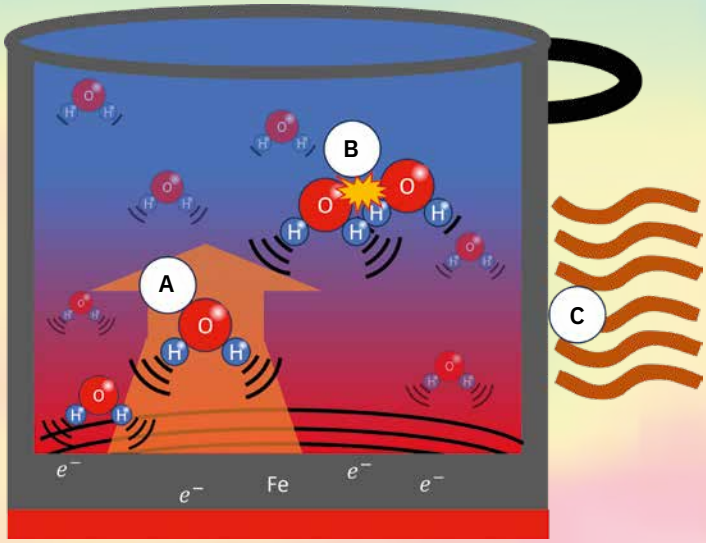


Figure 1: Types of heat transfer in a pot with hot water:
(A) Hot water rises in layers with colder water.
(B) Energy stored in vibrations and rotations is transferred to other water molecules by collisions.
(C) The hot surface of the pot emits heat radiation into the surroundings.

Heat transfer in packed beds

In packed beds, heat is transferred by particle contact, by the gas in the gaps and by radiation across free lines of sight. The effectiveness of heat transfer depends on a number of parameters, such as the conductivity and surface properties of the particles in the case of heat conduction and the emission and absorption characteristics in the case of heat radiation. A body that reflects well in the infrared range heats up more slowly than a body that absorbs most of the radiation.

A two-dimensional model for bulk material particles in a packed bed that do not have contact with each other can be realized as shown in Figure 2 by an arrangement of rods, where the center rod is heated and then emits infrared radiation into its immediate surroundings accordingly.

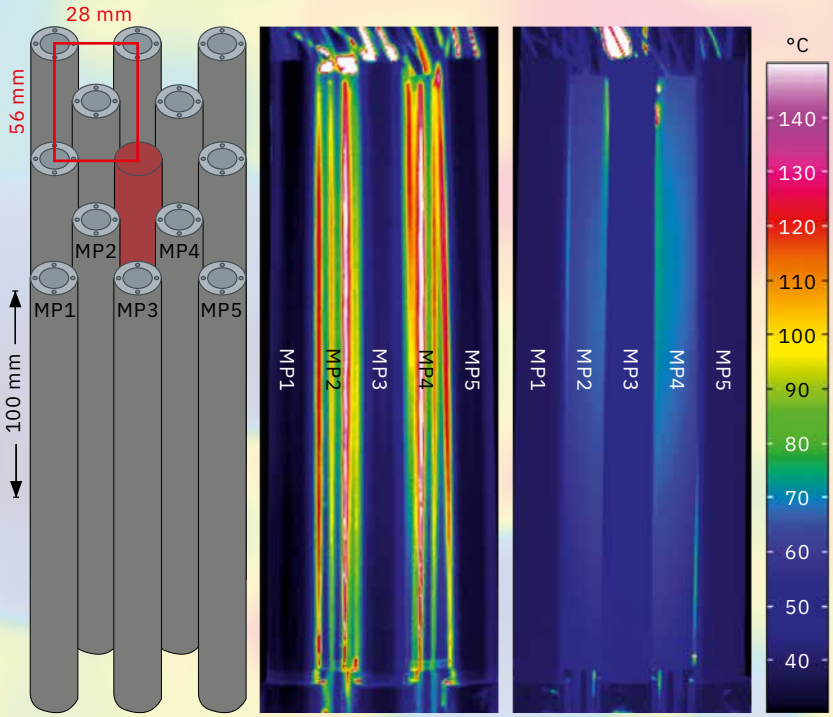


Figure 2: Model for investigating heat transfer in packed beds (left) and view of the indirectly heated rods made of stainless steel (center) and magnesium oxide (right) after 60 minutes. The rod marked in red has been heated to 600 °C. Modified from [1] and [2].

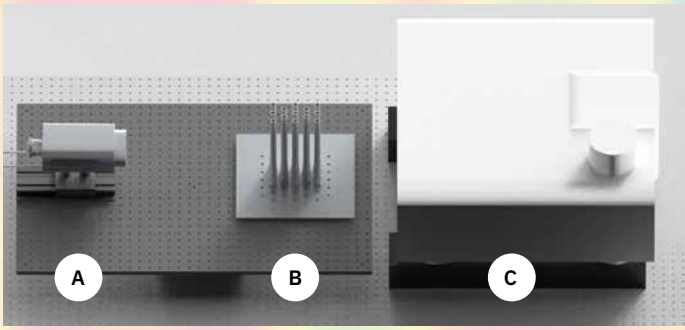


Figure 3: Experiment to validate the theoretical model:
(A) Thermal imaging camera.
(B) Metal rods as shown in Figure 2.
(C) FTIR spectrometer. Modified from [2].

A heatable rod (MP0) is surrounded by 12 unheated rods made of the same material. The aim is to heat the heatable rod to 600 °C and use temperature and FTIR measurements to determine how quickly and how much the surrounding rods are heated indirectly through the radiated heat. This process was simulated using the discrete ordinates method (DOM) and Monte Carlo ray tracing (MCRT).[1]

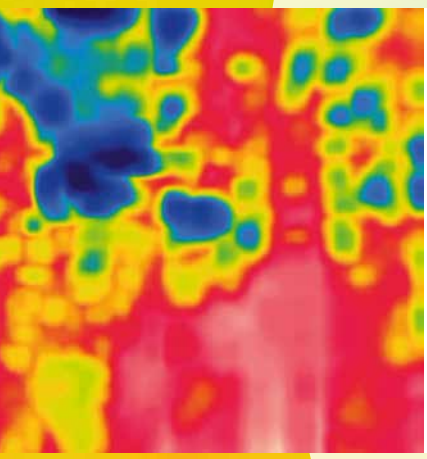
Experiment setup

The imaging measurement of thermal radiation is possible with infrared cameras, and the emission spectrum can be investigated using FTIR spectroscopy. Tyslik *et al.* designed the unique experimental setup shown in Figure 3, which is presented here in more detail, to verify the heat transfer simulations with real data.

The key element is the arrangement of rods (B) described above. The temperature of the indirectly heated rods is investigated using a thermal imaging camera (A). The infrared radiation emitted is coupled into a modified Shimadzu IRTracer-100, replacing the factory-fitted light source. The radiation passes through the Michelson interferometer and the empty sample chamber and is measured using an MCT detector. →

Results

The measurement results are described in detail in references [1] and [2] (see QR code at the end of this article). Stainless steel and magnesium oxide were used as the materials for the rods in this experimental investigation. A number of test parameters, such as the emissivity and the time required to reach the equilibrium temperature, were determined and discussed in detail. The central, directly heated rod radiates heat to the surrounding rods in the form of infrared radiation. This means these rods are heated indirectly and in turn emit heat in the form of infrared radiation but also reflect some of the infrared radiation.



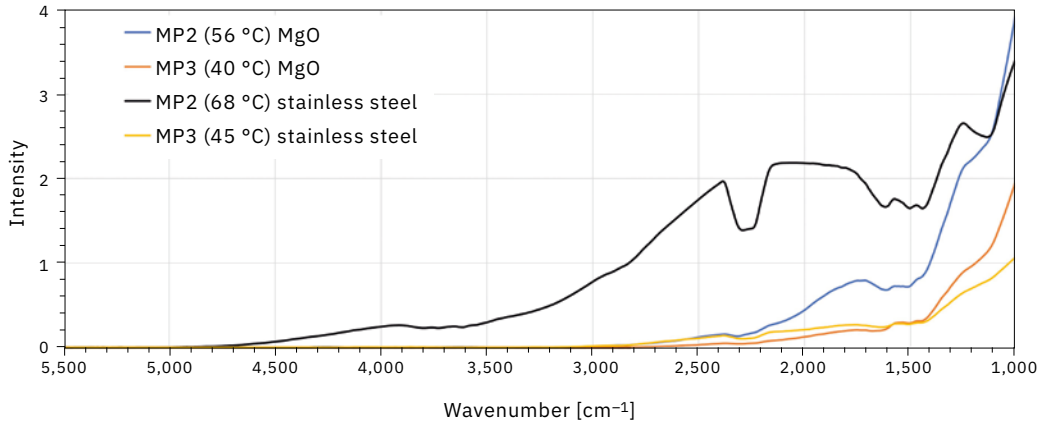


Figure 4: Comparison of emission spectra of the MP2 and MP3 rods, which are made of stainless steel and magnesium oxide

The emission spectra of MP2 and MP3 after 60 minutes at a target temperature of 600 °C are shown in comparison for both of the test materials in Figure 4. MP2 has a temperature of 68 °C (steel) and 56 °C (MgO). MP3 has a temperature of 45 °C (steel) and 40 °C (MgO).

As expected, the emission intensity of the warmer rod MP2 is significantly greater than that of the colder rod MP3. From 1,000 to 4,500 cm^{-1}, it is also significantly greater for stainless steel than for magnesium oxide. The emission spectrum of the stainless steel rod also shows a band from 1,600 to 2,500 cm^{-1}, which was not observed in this form for either of the magnesium oxide rods or MP3.

However, the measurement results were influenced by reflections from the rod surfaces, particularly in the case of rod MP2, which was positioned far inwards.

To investigate the effects of reflection, the emission spectrum of the heatable rod was measured at 67 °C in a setup without the surrounding rods. In this ex-

periment, the band observed in Figure 4 is not visible, as shown in Figure 5. This demonstrates just how much of the measured emission spectrum is caused by thermal radiation reflected from the surface. The results in [1] show that MCRT can reproduce the measurement results more accurately in simulations. However, the authors assume that in simulations of large real bulk materials, adaptation of the DOM is preferable as a computationally efficient compromise with adequate accuracy.[3] This approach will be validated against measurement results of the described setup.

Thermal conduction between the rods is prevented as far as technically possible in the setup described here, as the rods do not touch each other. In order to eliminate heat transfer by convection, a vacuum chamber will be added to the design in future.

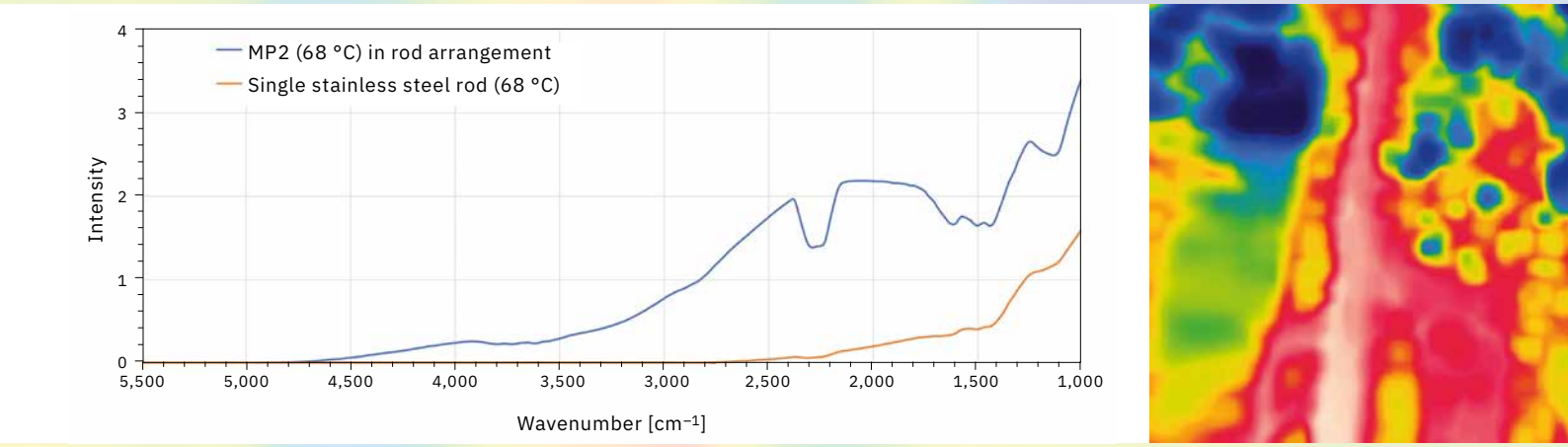


Figure 5: Comparison between the emission spectra of MP2 (stainless steel) measured in the composite (blue) and an insulated rod measured at the same temperature (orange)

It all comes down to reflection

The described experiment setup with an arrangement of metal rods and a modified FTIR spectrometer allows heat transport by infrared radiation to be investigated. In a first experiment, stainless steel and magnesium oxide were compared, and the relevance of the reflective properties of the material was demonstrated. Due to the significantly higher reflectivity of the steel rods, the heat radiation penetrates a collection of steel rods easier than a collection of magnesium oxide rods. These initial results and the comparison with computer simulations provide important incentives for further research and modeling of heat transfer in packed beds. Precise knowledge of the mechanisms of this heat transfer helps optimizing a wide range of process parameters in large-scale factories and, as a result, save a great deal of energy. Areas of application could be lime burning or iron processing.

Note

For more information and references, please refer to the digital version of this edition.

New ACCUTRACE™ Plus fuel marker BPE (butyl phenyl ether) fights crime

Robust and reliable GC-MS/MS method utilizing Shimadzu's MDGC switching technique

Waldemar Weber, Nerea Lorenzo Parodi, Ute Potyka, Xaver Mönnighoff, Shimadzu Europa GmbH

In January 2024, a new ACCUTRACE™ Plus fuel marker BPE (butyl phenyl ether) has replaced the previous "Solvent Yellow 124" standard in the European Union. Consisting of naphthenic hydrocarbons as solvent and BPE, the new marker is laundering-resistant, contains no harmful additives and is easy to handle. Moreover, it can be detected quickly via GC-MS at extremely low levels. Shimadzu prepared a sensitive, accurate and convenient GC-MS method according to the European regulation.

The new Euromarker and its predecessor

Since 2001, a common fuel marker has been used in the European Union (EU) as a fiscal marker to avoid not only confusion of differently taxed fuels and diesel but also to detect fuel fraud, dilution, adulteration and theft that lead to a loss of tax revenue for governments and oil companies in the EU of several billion EUR each year. In many European countries, fuels such as agricultural diesel, mineral diesel and heating oil are subsidized. To combat the misuse of the lower tax fuels, they were historically marked with different azo dyes called “Solvent Red”. The yellow marker “Solvent Yellow 124” was established for heating oil EU-wide from 2002 because it was easier to analyze. Under the name “Euromarker” it is added to fuels not intended for motor vehicles in amounts of 6 mg/L. Solvent Yellow 124 itself hardly colors the fuel oil, so still a red dye is also added for easy differentiation. It can be detected very quickly and sensitively with an indicator reagent even when strongly diluted, alternatively it can be quantified using high performance liquid chromatography (HPLC). However, the last review from the European commission showed that Solvent Yellow 124 is not that robust against common removal techniques. Smart cheaters even found a way for “fuel laundering”.

Laundering-resistant – no harmful additives – easy to handle

Furthermore, some European countries expressed concerns about the toxicity of azo dyes. Consequently, the European Commission decided to adopt ACCUTRACE™ Plus as a new, safer and more robust Euromarker. The new marker BPE (butyl phenyl ether) is laundering-resistant, contains no harmful additives and is easy to handle. Moreover, it can be detected quickly via GC-MS at extremely low levels. The decision for ACCUTRACE™ Plus fuel marker has come into effect in January 2024.

Sample preparation and calibration

A stock solution of BPE in xylene was used to prepare a 7-point calibration between 0.1 and 12 mg/L in diesel. The samples were analyzed directly without any sample preparation. Thanks to the heart-cut technique, most of the fuel matrix was transferred to the FID and only a very small part of the sample, containing the BPE, was directed to a second, polar column and finally to the mass spectrometer.

Results

A representative second dimension chromatogram of BPE in diesel, showing base line separation of BPE from diesel, can be seen in Figure 1. Excellent linearity was observed within the tested concentration range between 0.1 and 12 mg/L in two consecutive days, with a correlation coefficient of R2 > 0.9999, as can be seen in Figure 2. An LOD of 2.8 µg/L and an LOQ of 9.2 µg/L were achieved, allowing a detection of adulterated diesel far below the requested quantification limit. The results for all concentration levels are shown in Table 1. Recoveries between 93 % and 117 % were calculated in spiked diesel and gasoline samples, as can be seen in Table 2. →

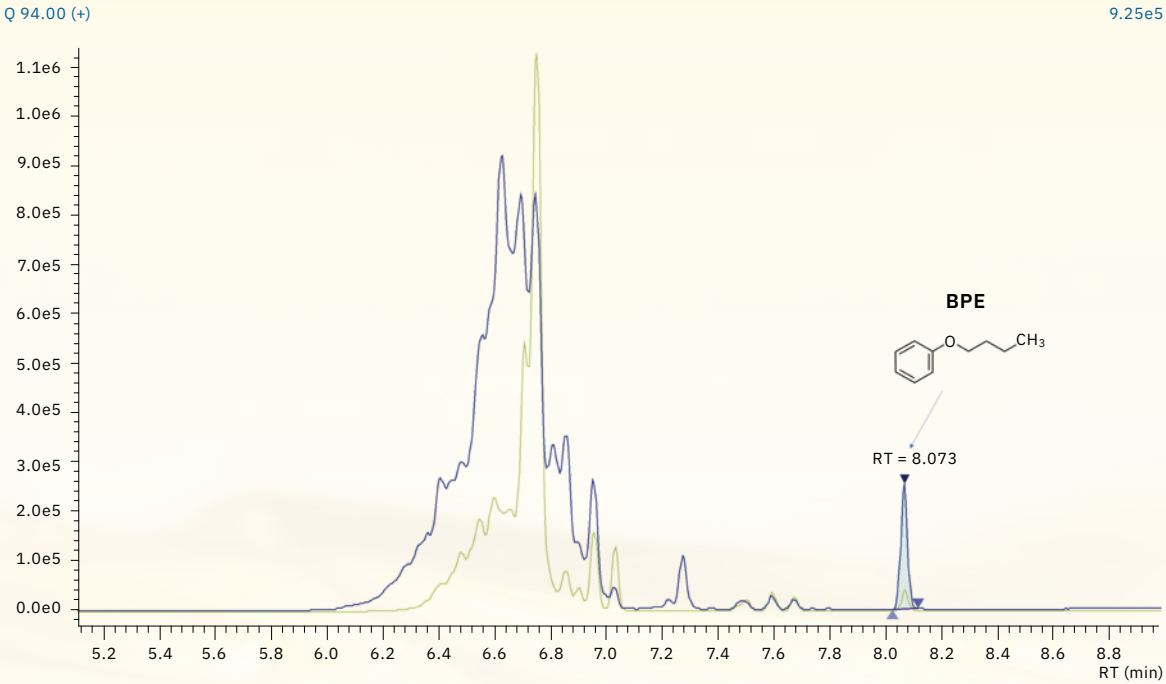


Figure 1: Second dimension chromatogram of 2 mg/L BPE in diesel, blue = target *m*/z 94, green = reference *m*/z 150

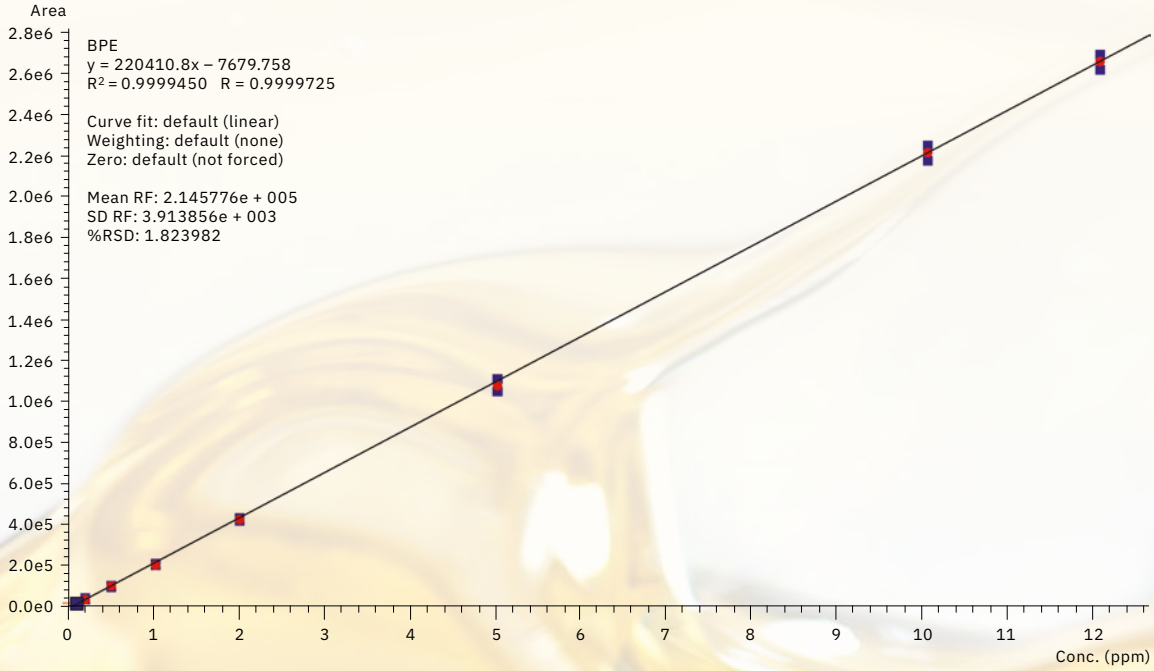


Figure 2: Interday linearity of BPE, concentration range 0.1–12 mg/L

	Repeatability in %		
Concentration [mg/L]	Day 1	Day 2	Interday
10	1.0	1.1	2.4
2	0.7	0.8	2.3
0.2	0.9	1.5	1.5
0.1	0.8	1.8	1.4

Table 1: Intraday and interday peak area repeatability of BPE in diesel at various concentrations

	Target conc. [mg/L]	Average conc. [mg/L]	RSD in %	Recovery in %
Diesel	0.2	0.21	1.9	106
	1.0	0.93	0.3	93
	7.46	6.92	0.2	93
Gasoline	0.2	0.23	0.8	117
	1.0	0.94	1.1	94
	7.46	7.23	0.7	97

Table 2: Quantification of spiked diesel and gasoline samples

Main unit	GCMS QP2020 NX with FID
Accessory	AOC-30i autosampler
Main consumables and columns	DB-17HT, 15 m × 0.25 mm × 0.15 µm; P/N 980-30834
	VF-WAXms, 30 m × 0.25mm × 1.0 µm; P/N 980-30835
	Retention gap, 2 m × 0.15 mm; P/N 980-10599
Software	GCMSsolution and LabSolutions Insight

Table 3: Proposed instrument setup

It can be observed that in both spiked samples BPE is well separated from the matrix. The recommended analytical hardware and software configuration is listed below in Table 3.

Minimized total analysis time – less downtime – increased overall throughput

The use of two-dimensional gas chromatography, in combination with an FID-2030 and a QP2020 NX enables the successful determination of BPE in diesel and gasoline. Thanks to this heart-cut system, not only the separation of the polar BPE from the non-polar fuel is achieved but the method can also be successfully validated. Furthermore, using this system results in a lower matrix fraction, reaching the second dimension and MS and therefore a drastically reduced ion source contamination. It also minimizes the total analysis time by allowing to backflush at the same time as the separation takes place in the second dimension. Because the system is more robust and the ion source needs to be cleaned less often, the downtime of the system is significantly reduced, increasing the overall throughput.

Note

For more information and references, please refer to the digital version of this edition.

Figure 3: GCMS-QP2020 NX equipped with an FID detector and an AOC-30i autosampler

Bisquaternary ammonium adduct with trifluoroacetate

MS^n fragmentation analysis of bisquaternary ammonium-trifluoroacetate adduct

Marta Kowalska, Dr. Remigiusz Bąchor,
Faculty of Chemistry, University of Wroclaw

Trifluoroacetate (TFA) is a mobile and persistent substance that is primarily introduced into the water cycle through the degradation of various fluorochemicals and remains in the environment in the long term. Also known as an ion-pairing agent, it reduces the signal intensity in the ESI-MS spectrum. Nonetheless, the examination of its binding by mass spectrometry has the potential to have a significant research impact on the formation of appropriate sensors.

In this study, we demonstrate the feasibility of identifying a stable bisquaternary adduct with trifluoroacetate, resulting in the formation of distinctive fragment ions in MS^n mode containing a covalently bound anion. This phenomenon requires bond reorganization, which leads to charge retention on the nitrogen atom. ⟶

The formation of adducts is a common occurrence in electrospray ionization-mass spectrometry (ESI-MS) analysis.[1] One common observation is the presence of potassium, sodium, ammonium or lithium adducts in the positive ion mode [2] and chlorine adducts in the negative ion mode.[3, 4] Trifluoroacetic acid may form adducts with positively charged analyte ions in the positive ion mode of ESI-MS, which reduces the signal intensity on the mass spectrum. This phenomenon is common due to the widespread use of TFA as an additive in high-performance liquid chromatography (HPLC). The utilization of TFA results in an enhancement of the chromatographic peak shape owing to the reduction of the silanol-group effect from columns.[5] For mass spectrometry analysis, it is important to understand the interaction between two associated molecules from a mechanistic and biological perspective. Certain compounds exhibit a low ionization efficiency during the ESI-MS experiment, thereby limiting their reliable identification. Moreover, as previously mentioned, the presence of anions, such as the TFA anion, may result in a decrease in signal intensity on the MS spectrum, which may pose a challenge during the analysis of certain compounds that are hardly ionized. The use of fixed charge tags, such as quaternary ammonium salts, is one of the approaches, which enhances the effectiveness of ionization and detection sensitivity.[6] Quaternary ammonium cations are nitrogen-containing ions with four aryl or alkyl chains attached to the nitrogen (NR_4^+ structure). Consequently, quaternary ammonium cations possess a constant positive charge.[7] The application of quaternary ammonium salts allows reducing the limitations of the ESI-MS/MS analysis due to the introduction of positive charge, thereby enabling the analysis of compounds with low ionization efficiency.[6]

Pyridinium group enhances ionization efficiency

Pyrylium salts are an example of a compound that has a constant positive charge due to the presence of a positively charged oxygen atom. The high reactivity of this atom toward nucleophiles has led to the use of pyrylium salts in the creation of pyridinium derivatives, compounds containing the quaternary ammonium atom. The presence of the pyridinium group, owing to its constant positive

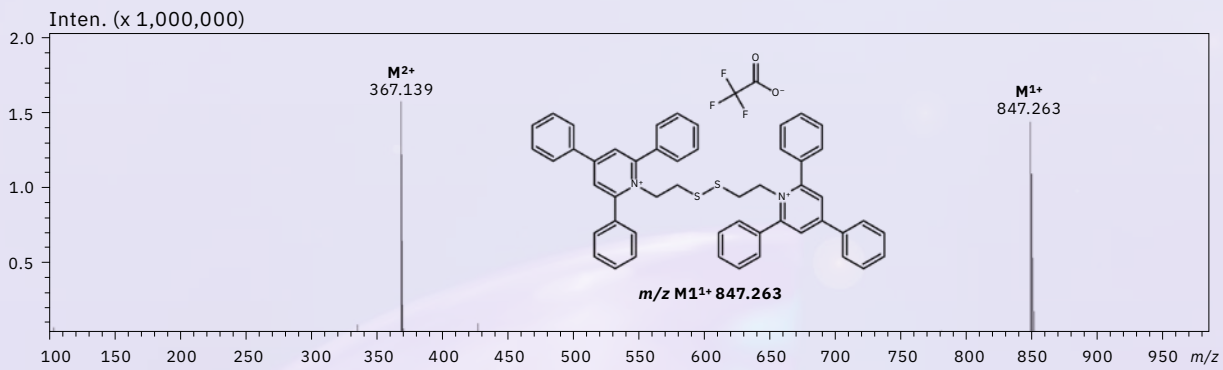


Figure 1: ESI-MS spectrum of (TPP)2-CYSTAM-TFA adduct in the positive mode. *m/z* range from 100 to 1,000.

charge, enhances the efficiency of ionization, resulting in enhanced sensitivity, such as in the detection of peptides.[8]

In the presented research, the gas phase formation of a non-covalent bisquaternary ammonium-trifluoroacetate adduct was described, even during an MS/MS experiment and monitored by Shimadzu LCMS-IT-TOF (Shimadzu, Kyoto, Japan).

The model bisquaternary ammonium compound in the form of 2,2'-disulfanediylbis(2,4,6-triphenylpyridinium) ((TPP)2-CYSTAM) was synthesized through the reaction between 2,4,6-triphenylpyrylium tetrafluoroborate and cystamine in the presence of *N,N,N*-triethylamine. The synthesized compound was purified using the reverse-phase HPLC, where the trifluoroacetic acid was used as mobile phase additive. The purified compound was then analyzed by ESI-MS (Figure 1). The analysis of (TPP)2-CYSTAM mass spectrometry in positive ion mode revealed the presence of the signal at *m/z* 367.139, which corresponds to the M^{2+} ion. The ESI-MS and ESI-MS/MS analysis confirmed the success of the synthesis.

Additionally, the ESI-MS spectrum (Figure 1) revealed the presence of the signal at *m/z* 847.263 ($M1^{1+}$). The isotopic pattern of the signal at *m/z* 847.263 ($M1^{1+}$) was practically the same as in the case of the signal at *m/z* 367.139 (M^{2+}). The detailed analysis showed that the signal at *m/z* 847.263 characterizes the +1 ion, which contains two sulfur atoms. The mass of the formed ion was found to be 113 Da higher than the mass of the bisquaternary ammonium compound (M^{2+} ion), which is characteristic to tri-fluoroacetate (112.986 Da). As it was mentioned above, TFA used in the mobile phase can cause suppression effects and decrease signal intensity. [5] However, the (TPP)2-CYSTAM has two positive charges, and during the formation of the (TPP)2-CYSTAM-TFA adduct only one positive charge is neutralized. Therefore, the formation of a singly charged non-covalent adduct is observed, which is stable in the gas phase. To check the stability of the (TPP)2-CYSTAM-TFA adduct, the ESI-MS/MS analysis was performed (Figure 2). ⟶

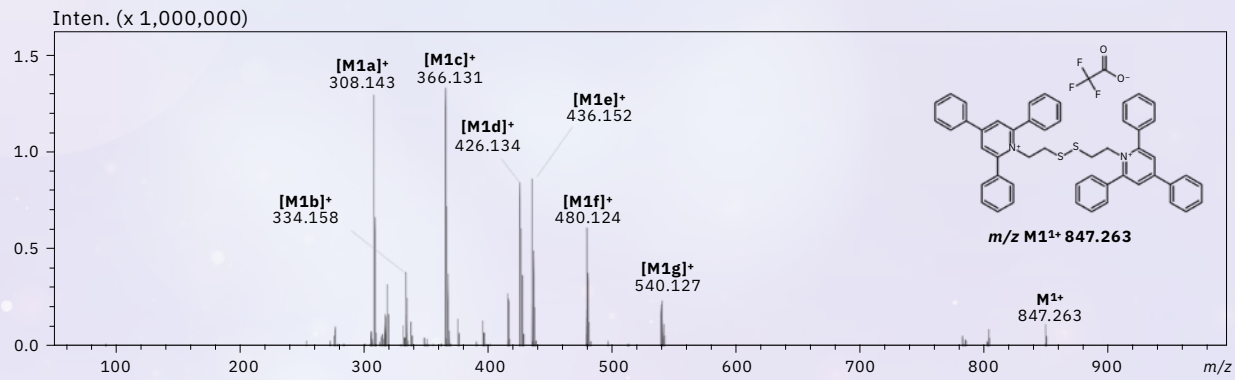


Figure 2: ESI-MS/MS spectrum of (TPP)2-CYSTAM-TFA adduct in the positive mode. Parent ion *m/z* 847.263, collision energy 50 %, *m/z* range from 50 to 1,000.

Performing the fragmentation of parent ion

In general, the adduct formed in ESI-MS does not show any other fragmentation than cation or anion loss in ESI-MS^n experiments. Therefore, the fragmentation of the parent ion at *m/z* 847.263 ($M1^+$) was performed. One of the most characteristic fragment ions was the $[M1a]^+$ ion at *m/z* 308.143, which corresponds to the protonated form of 2,4,6-triphenylpyridine (TPP). TPP is a commonly known reporter ion, which is formed during fragmentation of the TPP-modified peptides.[8] Apart from the $[M1a]^+$ ion, the fragmentation revealed the formation of the fragment ions $[M1b]^+$, $[M1c]^+$, $[M1d]^+$ at *m/z* respectively 334.158, 366.131 and 426.134, which were characteristic to dissociation of fragments of bisquaternary ammonium compound (Figure 2).

If the formed adduct is stable in the gas phase, the part of the molecule that remains with a positively charged quaternary nitrogen atom after dissociation should be neutralized by the presence of the trifluoroacetate anion. However, the obtained mass spectrum presents other signals that correspond to the fragment ions, which mass do not indicate the loss of the TFA moiety. The detailed analysis revealed that the signals characterizing ions at *m/z* 436.152 $[M1e]^+$, 480.124 $[M1f]^+$ and 540.127 $[M1g]^+$ are the TFA adducts with a singly charged nitrogen atom.

It is not straightforward to identify signals characterizing positively charged ions in the form of trifluoroacetate adducts, as it would require either additional charge formation or reorganization of chemical bonds. Furthermore, the *m/z* values of the signals depicted in the obtained ESI-MS/MS spectrum for fragment ions corresponding to the TFA adducts are shifted by 1 Da in comparison to the non-covalent form of the TFA adduct, which may indicate the occurrence of bond reorganization.

Laying the foundation for a future anion sensor for environmental samples

The MS/MS analysis showed that electrostatic interactions between bisquaternary ammonium compound and trifluoroacetate moiety can be converted into covalent bonds in the presence of collision energy. TFA anion forms the stable N-O bond with the bisquaternary ammonium cation (confirmed by computational methods based on DFT), which increase stability of the obtained adduct. The conversion to the covalent system requires bond reorganization and charge retention on the nitrogen atom. This occurrence makes it possible to observe the fragment ions containing the TFA moiety. The aforementioned interactions and the potential for their analysis in the future may result in the development of an anion sensor that can be utilized to determine the presence of certain anionic compounds in environmental samples.

Note
For more information and references, please refer to the digital version of this edition.

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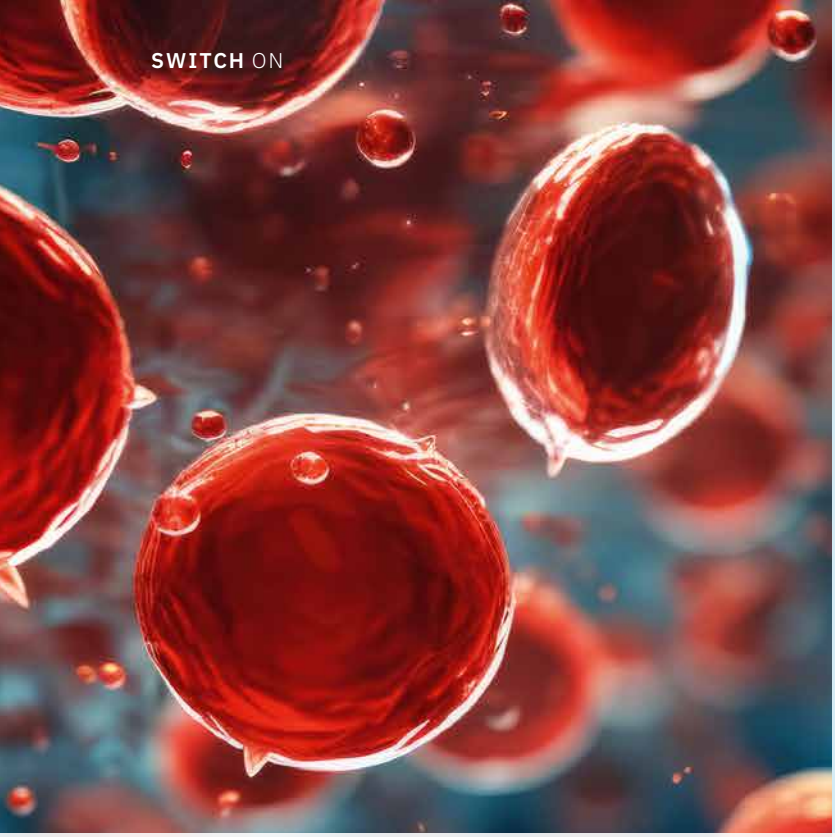
The author would like to thank Andrzej Reszka (Shim-Pol, Poland) for providing access to the Shimadzu LCMS-IT-TOF instrument.

New trump cards for blood analysis

DPS cards – a new method of collecting and analyzing blood

Dr. Martin Meyer, Shimadzu Europa GmbH

Many diseases these days can be diagnosed by analyzing blood samples. These methods, which are often very complex, place high demands on sample preparation. But in rural or underdeveloped areas in particular, these high requirements often cannot be met. Here it is important to obtain blood samples even under the most basic conditions, which lead to meaningful and reliable results in the laboratory. A challenge the blood plasma separation cards from Telimmune fulfill today. →



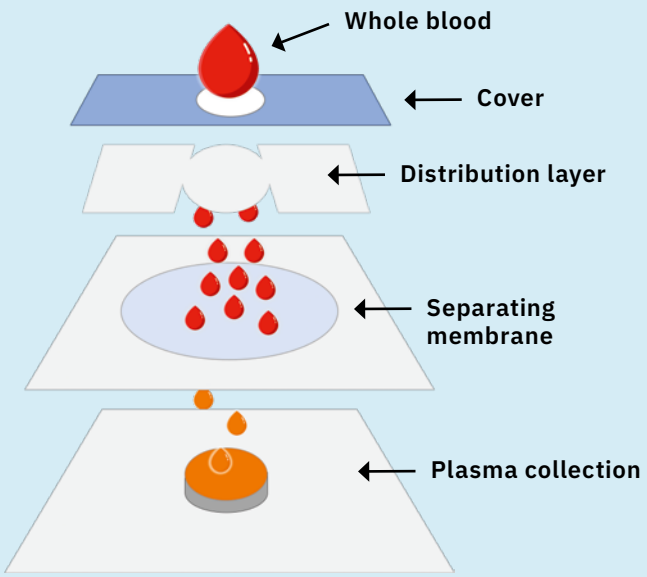


Figure 1: Separation of blood serum and blood plasma with Telimmune cards

Blood tests are carried out to diagnose a wide range of health conditions. These can provide valuable information about the function of organs, detect infections, reveal genetic disorders, determine hormone levels and help assess a person's overall state of health.

Traditional methods for blood collection vs. micro blood sampling

Venipuncture is the most widely used method of blood collection. Here, a needle is advanced into a vein, usually in the arm, to draw blood. This technique is preferred for larger quantities of blood and allows a variety of tests to be carried out from a single sample.

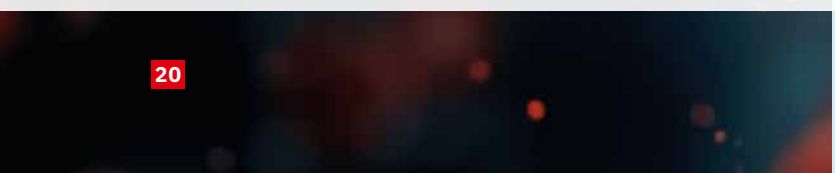
The finger prick method, also known as capillary blood collection, is a less invasive method that is often used for point-of-care testing. The fingertip is pricked with a lancet, and a small drop of blood is taken. This technique is often used to monitor blood glucose levels in diabetics. The lancing method is quick, convenient, and only a small amount of blood is required.

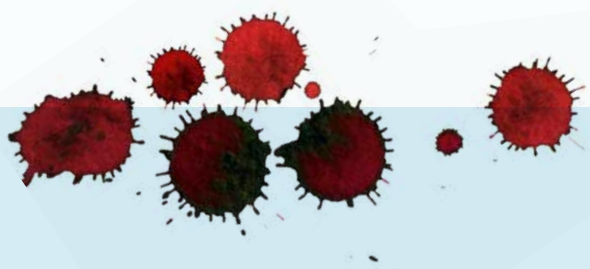
In contrast to the methods mentioned above, micro blood sampling is a relatively new technique that can be used to take very small amounts of blood, usually less than 100 microliters. The blood is collected in microtubes or micro collection cards, also known as dried blood spot cards or DBS cards for short. Dried blood spot cards in particular provide many advantages, including greater convenience for the patient, less pain and the ability to collect and store blood in remote locations. This is possible because the blood drops are dried, eliminating the need for low-temperature storage and transportation.

The advanced dried plasma spot cards (DPS cards)

The majority of clinical tests begin with blood collection, which requires a trained person (phlebotomist) and subsequently a laboratory to process the serum and plasma. Using dried drops of blood simplifies the procedure, as the two requirements mentioned above are not necessary, but this technique does have a few drawbacks (Table 1). For example, the cells in dried drops of blood can metabolize and react to coagulation factors.

The dried plasma separation cards developed by Telimmune (also known as dried plasma spot cards or DPS cards for short) separate the plasma from degraded and whole cells, eliminating many of the issues (Figure 1). In addition, they only collect a certain amount of plasma, regardless of hemocrit and viscosity. This makes the cards valuable for obtaining blood samples under the most basic conditions, which are still able to provide meaningful results in the laboratory. In Europe, the Telimmune cards are offered exclusively by Shimadzu. The cards were sold under the name Noviplex until the name change in 2021.





	Telimmune (DPS)	DBS cards	Whole blood microtubes	Intravenous blood tubes
Collection method	Finger prick or lancet	Finger prick or lancet	Finger prick or lancet	Needle
Blood volume	25–60 µL	Typical ≥ 100 µL	≥ 5 µL	≈ 20,000 µL
Assay	Plasma	Dried blood	Plasma or serum	Plasma or serum
Volumetric plasma collection	Yes, ≈ 3 µL per disk	X	X	Yes, but variable amounts
Storage and transport	Room temperature, dry	Room temperature, dry	≤ 6 °C, liquid	≤ 6 °C, liquid
Suitable for on-site sample collection	✓	✓	Low	X
Low sample preparation	✓	X	X	X
No biohazard	✓	✓	X	X
Regardless of hematocrit	✓	X	X	✓
No RBC interference	✓	X	✓	✓

Green = Product advantage Red = Product disadvantage Yellow = Neutral

Table 1: Comparison of the DPS method with other blood collection methods

Figure 2: Telimmune card Uno for collecting one plasma plate

A broad and versatile range of applications

Telimmune cards have been used for testing in an amplitude of applications, e.g. for ferritin, Helicobacter pylori (gastritis), vitamin D, HIV, genetic disorders, COVID-19, homocysteine, sickle cell anemia, warfarin, IgG antibodies and more.[2] Using previously published methods, more than 250 different components can be analyzed from the plasma cards. However, the potential of detectable components is over 2,000 analytes, providing ample possibilities for further usage.[1]

Collection

The area of skin that has just been cleaned with an alcohol swab is punctured with a lancing device. Two drops of blood are allowed to fall onto the spot marked on the card (Figure 2). After 3 minutes, the top layer of the card is removed. The removal disk underneath is air-dried for 15 minutes at room temperature. Afterwards or after storage, the plasma spot can be removed from the card and transferred to a vial for extraction.

Analytics

There are various methods for analyzing plasma. Approved techniques include ELISA, PCR, QPCR, enzymatics, chromatography, mass spectrometry and colorimetry. There are already a number of methods where Telimmune cards are combined with the analysis by Shimadzu liquid chromatography and mass spectrometry (LC-MS). Applications include the analysis of pesticides, protein biomarkers, warfarin, immunoglobulins, steroids and the control and monitoring of therapeutic drugs. Moreover, the plasma collection plates have a diameter of 6.4 mm. This allows an optimal fit for the use of flat-bottomed 96-well plates, facilitating automated sample preparation by using an autosampler.

Telimmune cards are the trump cards for the future

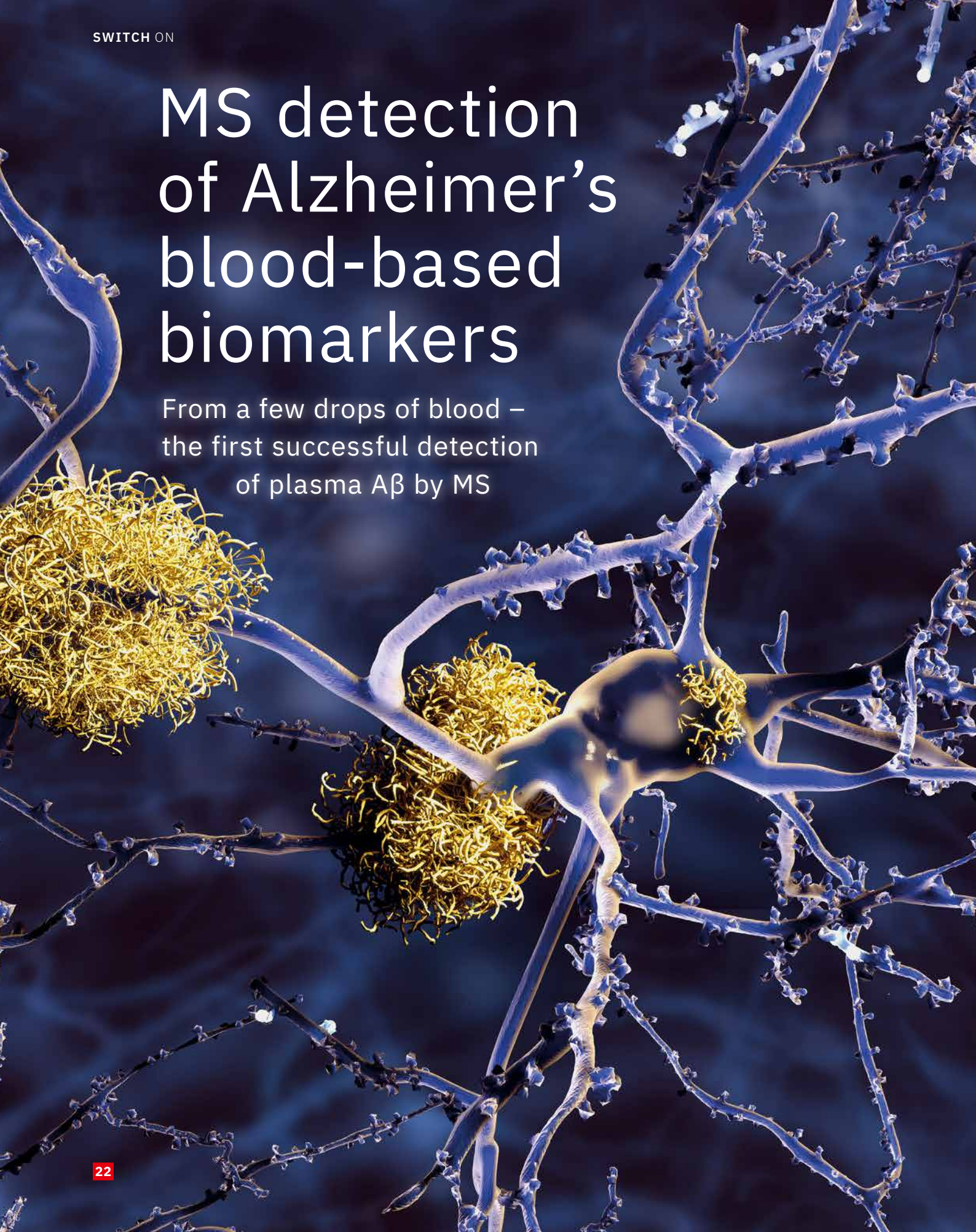
Overall, collecting blood using Telimmune plasma collection cards offers a convenient and minimally invasive method of blood collection, storage and analysis that is beneficial in various clinical and research settings and others yet to be tried and tested. Telimmune cards hold all the cards for the future.

Note

For more information and references, please refer to the digital version of this edition.

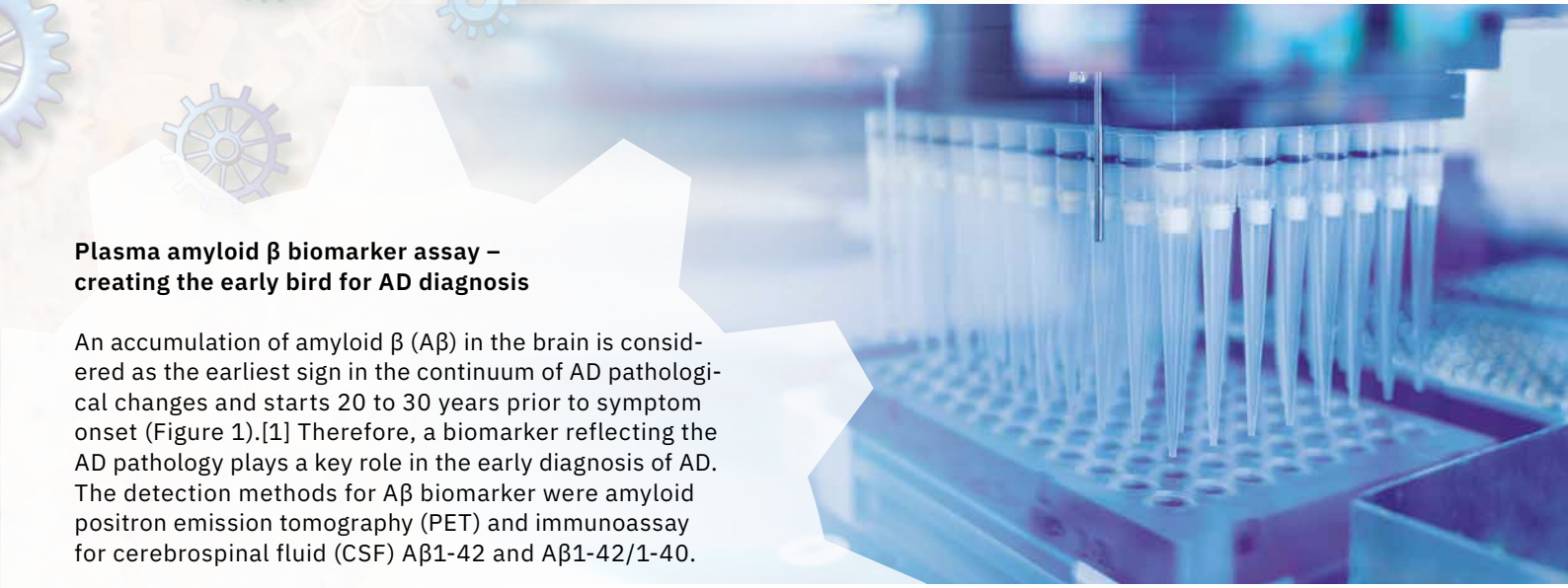
MS detection of Alzheimer's blood-based biomarkers

From a few drops of blood – the first successful detection of plasma Aβ by MS



Dr. Naoki Kaneko, Shimadzu Corporation

Amyloid β (Aβ) accumulation in the brain is considered as the earliest sign of Alzheimer's disease (AD) pathology. Recently developed disease-modifying therapies (DMTs) target patients with early-stage AD, hence blood-based biomarkers are needed for a detection of Aβ accumulation. We combined immunoprecipitation (IP) with mass spectrometry (MS) to develop an IP-MS technique that resulted in the first successful detection of plasma Aβ by MS. This technique revealed a high concordance between a composite biomarker of two plasma Aβ ratios and amyloid PET status. As advances are made in DMTs, blood-based biomarkers will become increasingly valuable due to their key role for screening patients, monitoring drug effects and diagnosis.



Plasma amyloid β biomarker assay – creating the early bird for AD diagnosis

An accumulation of amyloid β (Aβ) in the brain is considered as the earliest sign in the continuum of AD pathological changes and starts 20 to 30 years prior to symptom onset (Figure 1).[1] Therefore, a biomarker reflecting the AD pathology plays a key role in the early diagnosis of AD. The detection methods for Aβ biomarker were amyloid positron emission tomography (PET) and immunoassay for cerebrospinal fluid (CSF) Aβ1-42 and Aβ1-42/1-40.

However, amyloid PET requires a large PET equipment and is expensive, and CSF Aβ causes more invasiveness of collecting CSF. Therefore, there was a need for a blood-based biomarker that can be measured easily. In recent years, the clinical utility of plasma biomarkers has been reported with the significant technological advancements for plasma biomarker measurement. Main technologies that measure plasma Aβ are an immunoprecipitation coupled with mass spectrometry (IP-MS) and a sandwich immunoassay employing two kinds of antibodies. A head-to-head study for comparison of plasma Aβ assays has reported a higher accuracy in IP-MS than in the immunoassay.[2, 3, 4]

In the following passage, amyloid MS technique using IP-MS methodology is highlighted with matrix-assisted laser desorption/ionization time of flight MS (MALDI-TOF MS) and high performance of plasma Aβ biomarkers in clinical studies. ⟶

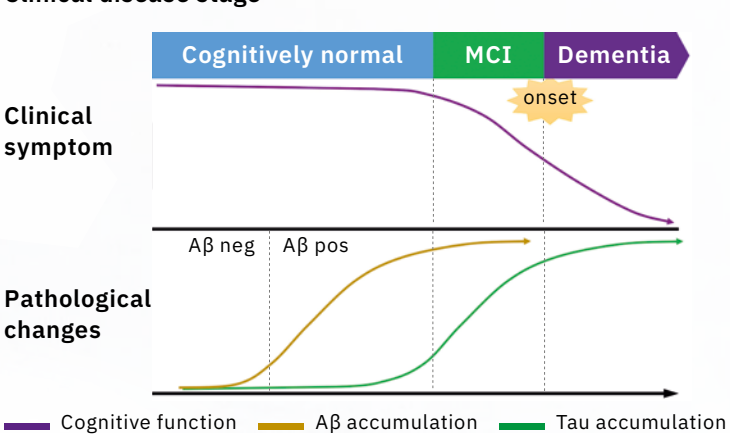
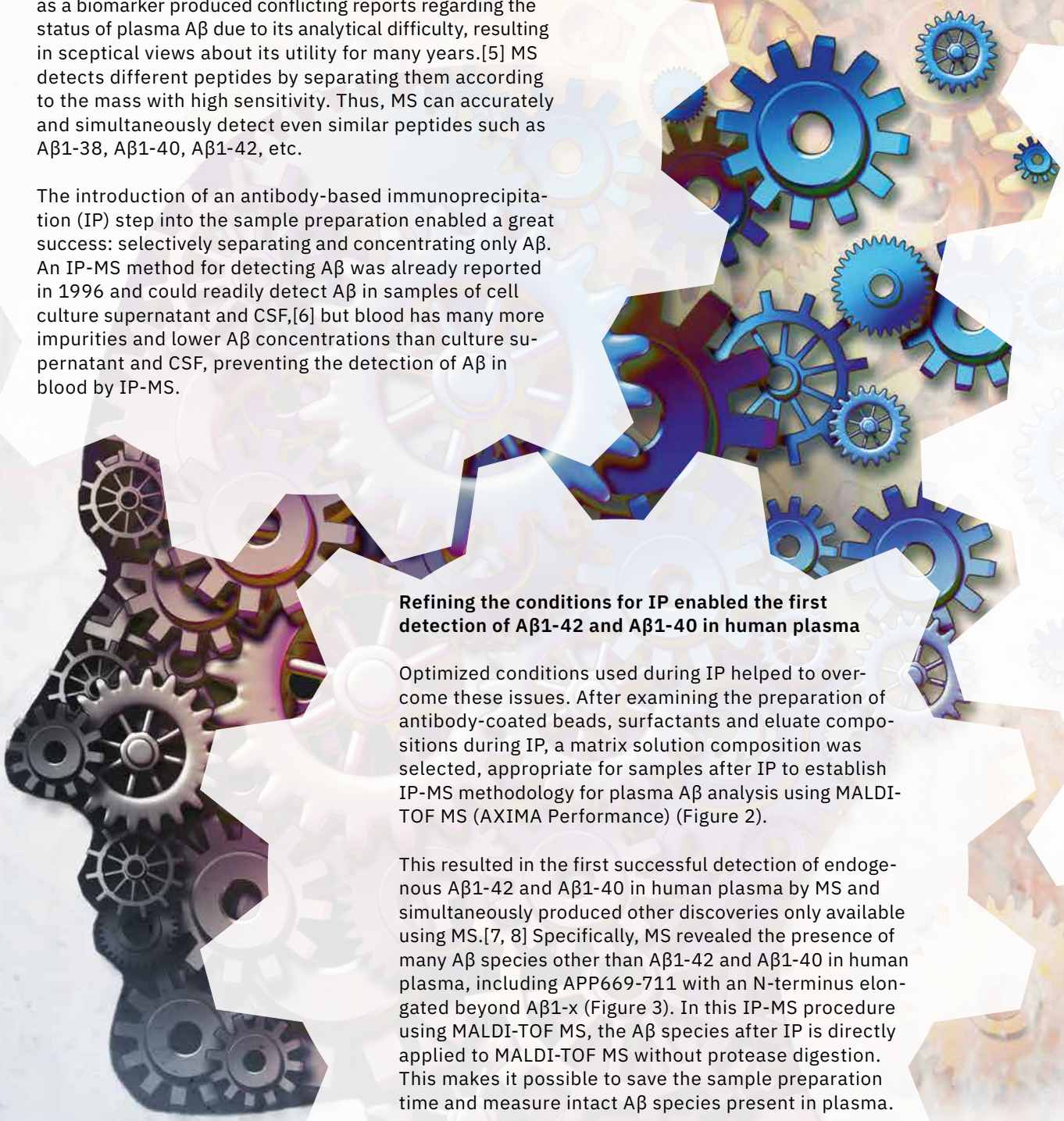


Figure 1: AD progression model

Amyloid MS: IP-MS methodology using MALDI-TOF MS

Immunoassays have been used in plasma Aβ biomarker research since 1996. However, many studies of plasma Aβ as a biomarker produced conflicting reports regarding the status of plasma Aβ due to its analytical difficulty, resulting in sceptical views about its utility for many years.[5] MS detects different peptides by separating them according to the mass with high sensitivity. Thus, MS can accurately and simultaneously detect even similar peptides such as Aβ1-38, Aβ1-40, Aβ1-42, etc.

The introduction of an antibody-based immunoprecipitation (IP) step into the sample preparation enabled a great success: selectively separating and concentrating only Aβ. An IP-MS method for detecting Aβ was already reported in 1996 and could readily detect Aβ in samples of cell culture supernatant and CSF,[6] but blood has many more impurities and lower Aβ concentrations than culture supernatant and CSF, preventing the detection of Aβ in blood by IP-MS.



Refining the conditions for IP enabled the first detection of Aβ1-42 and Aβ1-40 in human plasma

Optimized conditions used during IP helped to overcome these issues. After examining the preparation of antibody-coated beads, surfactants and eluate compositions during IP, a matrix solution composition was selected, appropriate for samples after IP to establish IP-MS methodology for plasma Aβ analysis using MALDI-TOF MS (AXIMA Performance) (Figure 2).

This resulted in the first successful detection of endogenous Aβ1-42 and Aβ1-40 in human plasma by MS and simultaneously produced other discoveries only available using MS.[7, 8] Specifically, MS revealed the presence of many Aβ species other than Aβ1-42 and Aβ1-40 in human plasma, including APP669-711 with an N-terminus elongated beyond Aβ1-x (Figure 3). In this IP-MS procedure using MALDI-TOF MS, the Aβ species after IP is directly applied to MALDI-TOF MS without protease digestion. This makes it possible to save the sample preparation time and measure intact Aβ species present in plasma.

→

Amyloid β (Aβ) sequence

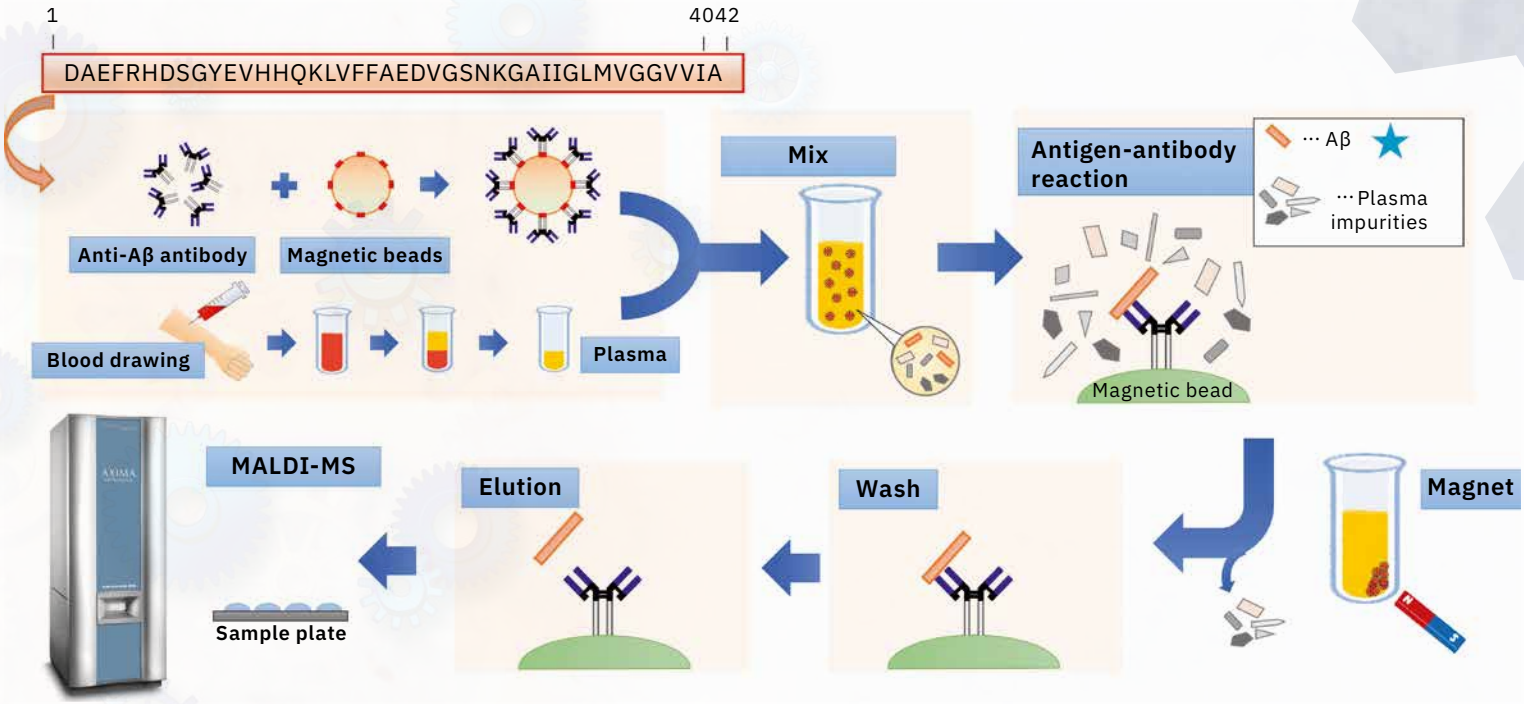


Figure 2: Amyloid MS: IP-MS methodology for plasma Aβ peptides

Amyloid precursor protein (APP)

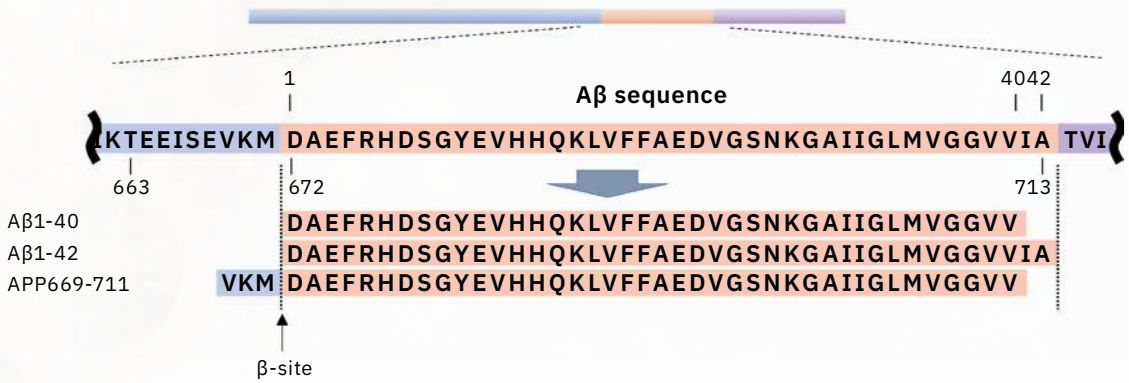
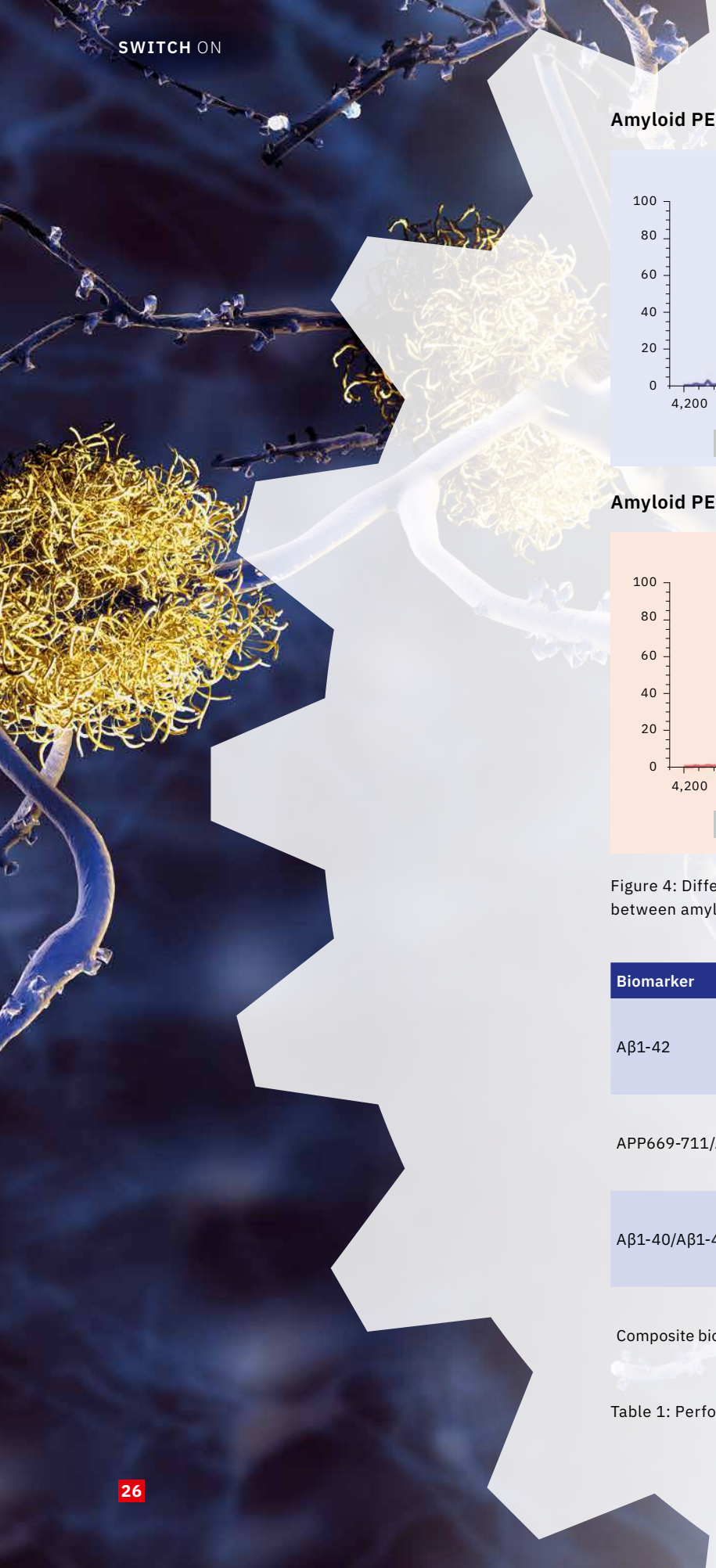
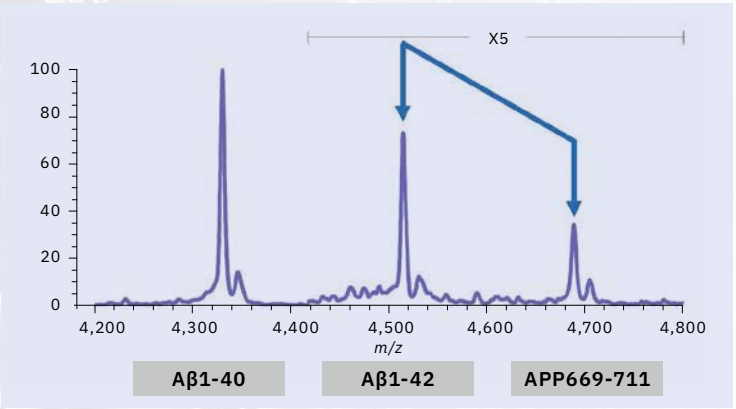


Figure 3: Plasma Aβ peptides detected by IP-MS



Amyloid PET-negative (cognitively normal)



Amyloid PET-positive (Alzheimer's disease)

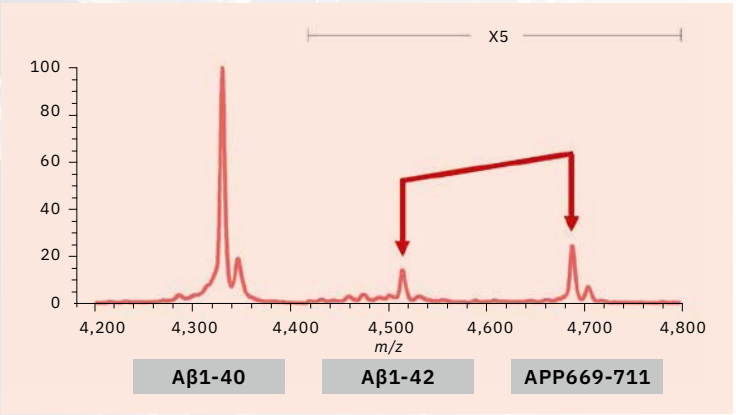


Figure 4: Difference of plasma Aβ peaks on MALDI-TOF mass spectra between amyloid PET-positive and PET-negative cases

Biomarker	Evaluation	NCGG (n = 121)	AIBL (n = 111)
Aβ1-42	AUC	87.2 %	75.7 %
	Sensitivity	74.0 %	78.3 %
	Specificity	88.7 %	66.7 %
	Accuracy	82.6 %	73.0 %
APP669-711/Aβ1-42	AUC	92.3 %	89.5 %
	Sensitivity	68.0 %	86.7 %
	Specificity	91.5 %	74.5 %
	Accuracy	81.8 %	81.8 %
Aβ1-40/Aβ1-42	AUC	96.7 %	88.9 %
	Sensitivity	96.0 %	90.0 %
	Specificity	87.3 %	70.6 %
	Accuracy	90.9 %	81.1 %
Composite biomarker	AUC	96.7 %	94.1 %
	Sensitivity	86.0 %	91.7 %
	Specificity	88.7 %	82.4 %
	Accuracy	87.6 %	87.4 %

Table 1: Performance of plasma Aβ biomarkers

Extensive blood-based biomarker studies

The research for an Aβ biomarker by IP-MS commenced in 2013 in cooperation with Japan's National Center for Geriatrics and Gerontology. This biomarker discovery study using IP-MS and amyloid PET imaging with PIB (62 cases) detected a higher ratio of APP669-711 to Aβ1-42 (APP669-711/Aβ1-42) in plasma from amyloid PET-positive patients (Figure 4).[9] Receiver operating characteristic (ROC) analysis of the APP669-711/Aβ1-42 ratio for amyloid PET-positive patients also showed a high area under the curve (AUC) value of 96.9 %. The APP669-711/Aβ1-42 ratio was also significantly correlated with the standardized uptake value ratio (SUVR) of amyloid PET, with a correlation coefficient of 0.687 ($p < 0.001$). Based on this data, a high concordance between plasma APP669-711/Aβ1-42 ratio and amyloid PET was reported for the first time in 2014.

A validation study was conducted in two datasets from Japan's National Center for Geriatrics and Gerontology and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Aging (AIBL). ROC analysis of the APP669-711/Aβ1-42 ratio for amyloid PET-positive cases (using the PIB tracer) revealed a high AUC value of 92.3 % in the Japanese dataset (121 cases) and 89.5 % in the Australian dataset (111 cases) (Table 1).[10] A composite biomarker created by combining APP669-711/Aβ1-42 ratio and Aβ1-40/Aβ1-42 ratio (a well-known biomarker) also improved AUC values to 96.7 % in the Japanese dataset and 94.1 % in the Australian dataset. The concentration of Aβ1-42 in CSF is known to decrease when Aβ accumulates in the brain, a phenomenon that was similarly identified in the data, with reduced plasma levels of Aβ1-42 in amyloid PET-positive cases. This change of Aβ1-42 may be caused by Aβ1-42 being trapped in the brain, which lowers the amount of Aβ1-42 available to enter bodily fluids. This report published in 2018 showed a high concordance between plasma Aβ measured by IP-MS and amyloid PET and served as a trigger for the recognition of blood Aβ as a useful biomarker by researchers worldwide.

Note

For more information and references, please refer to the digital version of this edition.

Less invasive biomarkers reflecting AD pathology lead the way to AD drug discovery

Amyloid MS technique can measure unique APP669-711/Aβ1-42 ratio as well as Aβ1-40/Aβ1-42 ratio in plasma. The Aβ composite biomarker created by combining the two Aβ ratios demonstrated high accuracy in distinguishing amyloid PET-positive cases from amyloid PET-negative cases. Biomarkers that reflect AD pathology are essential to AD drug discovery, diagnosis and staging, and blood-based biomarkers that provide a less invasive means of measuring large numbers of specimens will become increasingly useful.

Low carbon, big impact: making the most of biowaste

Making useful materials from biological waste streams using pyrolysis

Dr. Daniel Nowakowski, Aston University, Energy and Bioproducts Research Institute

Pyrolysis of bio-derived waste has the potential to provide a lower-carbon, more sustainable way of producing chemicals that are currently made from petrochemicals. But with biowaste feedstocks being so diverse, how can we determine the best use of a particular resource? We talk to Dr. Daniel Nowakowski at Aston University's Energy and Bioproducts Research Institute (EBRI) about the challenges faced when thermally processing biowaste – and how collaborating with Shimadzu is helping him and his team to maximize the potential benefits of diverse feedstocks.

The diverse composition of biowaste

Society has long used biowaste in the form of compost for improving or fertilizing soils, or for releasing heat by burning. But in the last 20 years, there has been growing realization that biowaste can also be used to generate useful chemicals by subjecting it to high temperatures in the absence of air (known as pyrolysis).

These products can be very varied, says Dr. Daniel Nowakowski, an expert in pyrolysis at Aston University, UK: *"By heating up biowaste in a carefully controlled fashion, we can generate solids, liquids and gases with a wide range of interesting uses."* →

Figure 1: Adam Elaradi (Undergraduate Researcher) and Dr. Sarah Asplin (Postdoctoral Research Associate) operate the laboratory-scale intermediate pyrolysis system at EBRI. The system can take up to 300 g/h of biomass and has a maximum processing temperature of 600 °C.

Investigating feedstock to derive the maximum benefit

As Lecturer in Chemical Engineering and Applied Chemistry at Aston's Energy and Bioproducts Research Institute (EBRI), Dr. Nowakowski has first-hand knowledge about the types of biowaste sources and the ways in which the resulting products can be used – from carbon-storing soil conditioners to sustainable fuels.

This knowledge is put to use every day, not just through the team's fundamental research but through their project work for government agencies, organizations and companies looking to do more with their biowaste. When they're given a biowaste sample to investigate, he says, we ask one question: *"How can we tailor the pyrolysis process to derive products with the greatest value or benefit?"*

Pyrolysis – process and products

Before going further, it's worth first understanding what pyrolysis is. Otherwise known as "heat treatment", pyrolysis involves raising the temperature of a material in a reactor to 400–600 °C in the absence of oxygen. The material needs to be fairly dry, with typically less than 20 % water, to avoid compromising energy efficiency and causing phase separation of pyrolysis liquids.

"This closely mirrors the age-old process of making charcoal," says Dr. Nowakowski, *"but with much greater control over the conditions."* Also, he adds, nothing goes to waste: *"Unlike in conventional charcoal production, we're not just interested in the solid matter left behind – we also capture all the vapours and gases emitted in order to extract value from them too," he explains.*

The pyrolysis process leads to the production of three classes of materials:

- **Biochar** is a highly porous form of carbon and typically takes the form of a black powder. It's primarily of interest as a soil conditioner that can store carbon in the soil for extended periods, in a process known as carbon sequestration and storage (CCS).
- **Bioliquids** contain a complex mix of carboxylic acids, phenols (and their derivatives), esters, ketones, anhydrides, ethers and oxygen-containing heterocycles. During pyrolysis, they're released as vapours but are subsequently condensed into liquids. The water-miscible (aqueous) fractions, because of their acid content, are also known as "wood vinegar", which has attracted interest as a fungicide and plant growth enhancer.
- **Pyrolysis gas** (also called "pyrogas") includes methane, ethane and other hydrocarbons up to C5, hydrogen, carbon monoxide and traces of carbon dioxide. Gaseous hydrocarbons can be used to fuel the pyrolysis reactor, while hydrogen and carbon monoxide (syngas) can be used to produce ammonia or methanol. Hydrogen is also of interest as a "green" fuel for transport, particularly for heavy goods vehicles (for which electrification is not currently practical).

Tailoring the pyrolysis process

The amounts of biochar, bioliquids and biogases produced vary greatly depending upon the volatile content of the original material and the processing conditions, says **Dr. Sarah Asplin**, a Research Associate at EBRI, who is using her knowledge and skills from her Ph.D. in the field of biomass pyrolysis. She explains that slow, relatively low-temperature pyrolysis of biomass in a batch reactor might yield up to 50 % biochar and 25 % each of bioliquid and biogas. In contrast, quick, high-temperature pyrolysis of high-quality woodchip in a fluidized-bed reactor could result in up to 75 % bioliquid, with most of the remainder being gases and very little biochar.

Along with feedstock quality, all these factors feed into decisions about what to do with a particular feedstock, says Dr. Asplin: *"For example, clean feedstocks, especially those that are lignin-rich, are generally best turned into biochar because of the low loadings of contaminants in the final products, whereas low-quality feedstocks are better suited to bioliquid or biogas, because clean-up by liquid-liquid separation, distillation or downstream processing is a necessary part of producing them."*

Quick initial analysis – combining the right methods

To properly understand the potential of biomass and other organic waste requires an in-depth study of their chemical composition, says Dr. Nowakowski: *"You can't simply get away with applying a single analytical method!"*

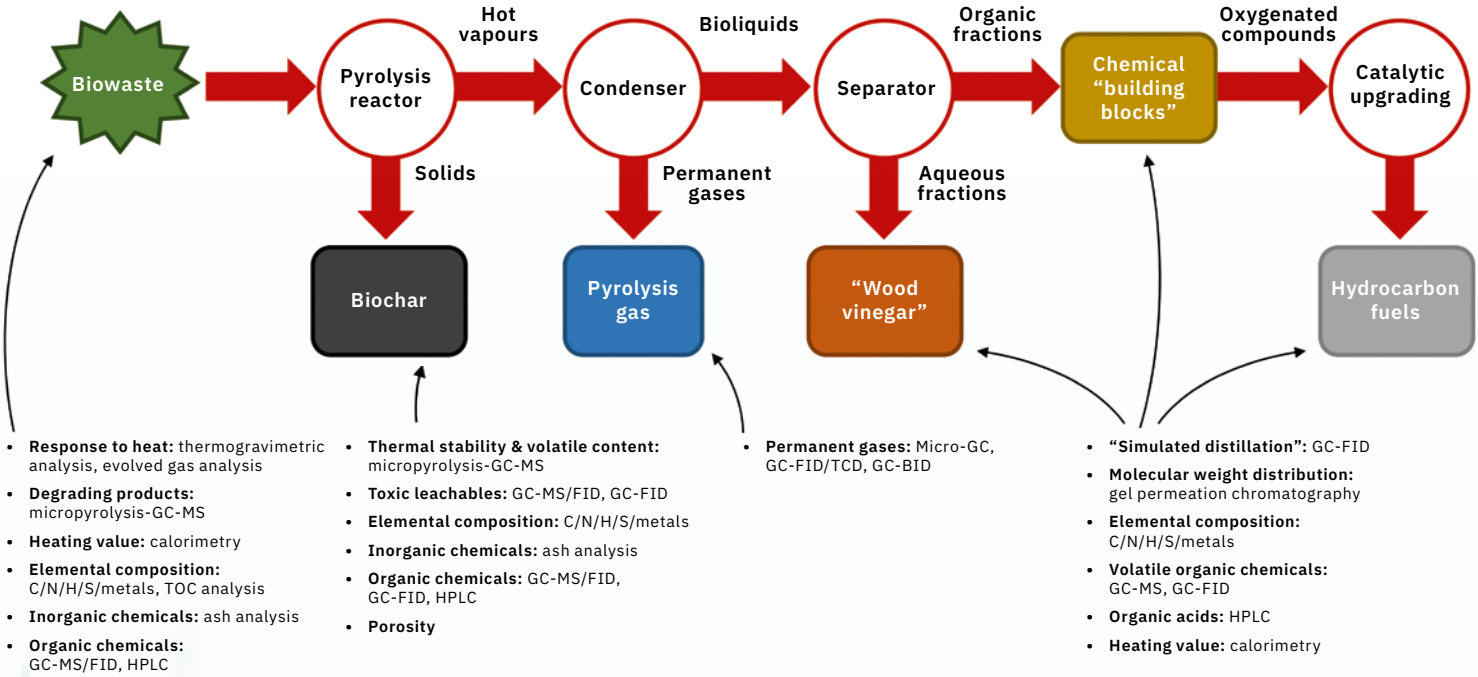


Figure 2: The analytical framework for pyrolysis of biowaste used at EBRI, showing the main stages (circles) and products (rectangles) and the analytical processes used at each stage

One of the first steps when understanding the potential of a new material, he explains, is to carry out a **thermal degradation** study. This involves heating a small portion (about 10 mg) up to about 750 °C in a thermogravimetric analyzer and monitoring how the mass changes as the temperature rises, giving the researchers an initial idea of its volatile content and solid residue. At the same time, the gases released can also be sent straight to a detector using a technique known as **evolved gas analysis**, again to provide a first impression of what the sample contains.

More information on the products obtained from thermal degradation happens through **micropyrolysis-GC-MS**. This uses what is in essence a miniature version of the full-scale pyrolysis equipment, with a GCMS fitted to the outlet, configured to analyze the vapour-phase products.

Figure 3: Anitta Xavier (Research Placement Student) injects a biomass sample into a Frontier Lab micropyrolyzer for direct transfer to the Shimadzu GCMS-QP2010 SE system

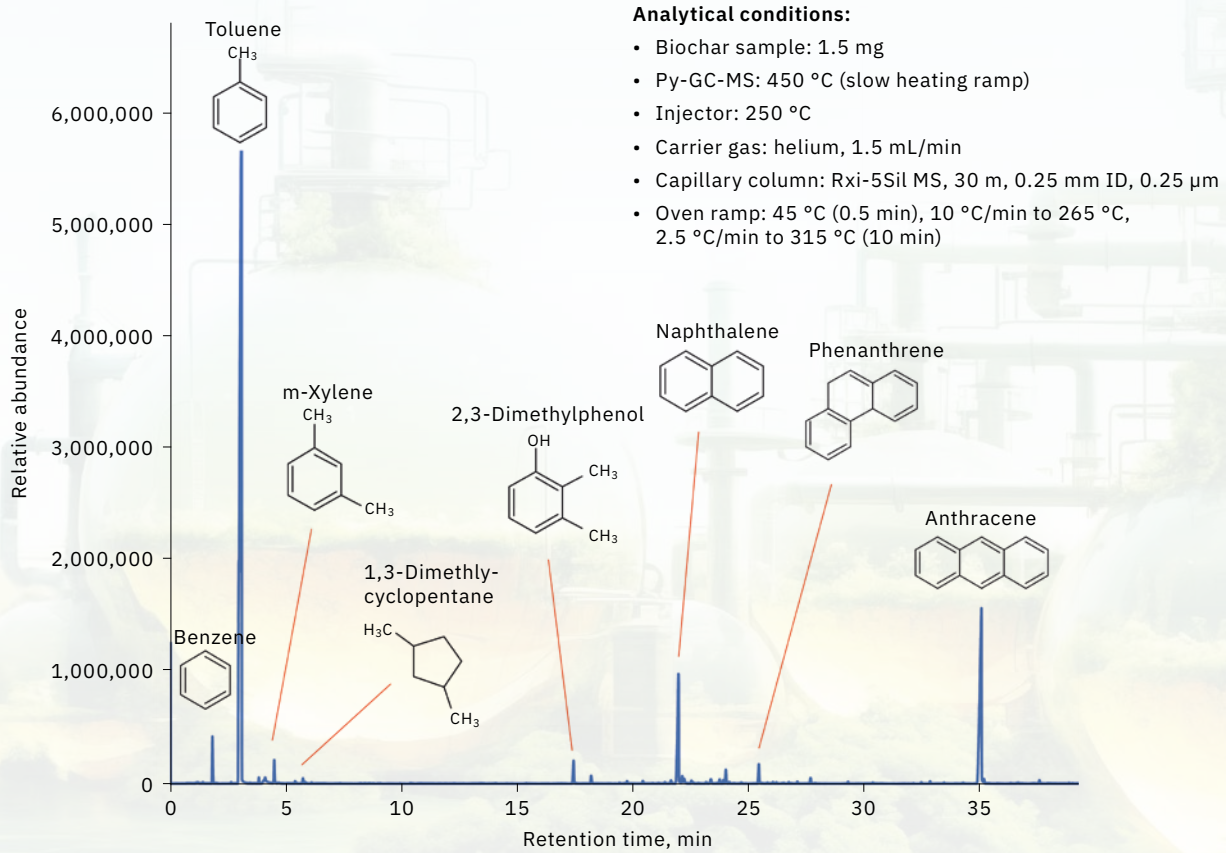
The advantage of this setup, says Dr. Nowakowski, is its speed. *"In just an hour, you can complete the preparation and analysis of a test sample and get an idea of the vapours that could be obtained from the feedstock,"* he explains. By adding an internal standard, the amounts of given products can be quantified and a preliminary assessment of product yields obtained, he adds.

The next steps depend on the outcome of those initial investigations, says Dr. Nowakowski, but they will often employ **elemental analysis** or **TOC analysis** to find the "total organic carbon" present in the sample. Another method useful at this point is **calorimetry** to find the heating value.

Biochar as a soil improver and polymer additive

One project with clear benefits on the ground, he says, is a collaboration with Birmingham City Council. This involves generating a biochar-based **soil improver** from the large amount of tree and shrub cuttings that the council produces from the parks and gardens that it maintains. Called the "Urban Biochar and Sustainable Materials Demonstrator" project, the cuttings are taken to the council-run Cofton Nursery in Longbridge (Birmingham, UK) and first screened to remove leaves and other compostable material. The larger, more woody material is shredded and pyrolyzed on-site in a unit installed in a shipping container, yielding biochar. This is used in various projects locally to improve soil structure and boost plant growth while also incorporating the carbon in a form that is not readily biodegraded and so providing a role as a "carbon sink".

Another use of biochar that Dr. Nowakowski's team is investigating relates to its use as **a filler in plastic composites** used (for example) in 3D printing. This is relevant because, as he explains, biochar isn't always 100 % carbon: *"Depending on the feedstock and process conditions, cold spots can arise in the biochar, which can trap small amounts of low-volatility compounds such as polycyclic*



Analytical conditions:

- Biochar sample: 1.5 mg
- Py-GC-MS: 450 °C (slow heating ramp)
- Injector: 250 °C
- Carrier gas: helium, 1.5 mL/min
- Capillary column: Rxi-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm
- Oven ramp: 45 °C (0.5 min), 10 °C/min to 265 °C, 2.5 °C/min to 315 °C (10 min)

Figure 4: A typical example showing the results of analyzing a sample of biochar by GC-MS to assess its purity. In this case, the compounds highlighted would be undesirable in the final product because of their known toxicity; work carried out at EBRI aims to understand the pyrolysis conditions that lead to these contaminants and minimize them. The analysis used a Frontier Lab EGA/PY-3030 double-shot analytical pyrolyzer, close-coupled with a Shimadzu GCMS-QP2010 SE system.

aromatic hydrocarbons, which are toxic. These would normally remain bound to the biochar but if you're using it as a filler in plastic, which then ends up being heated during processing, it could release these chemicals and cause a safety issue."

To study this effect, he adds, the researchers use the "pyroprobe" setup to assess the thermal stability of small samples of biochar-modified plastic pellets to be sure that the product is going to be safe for the purpose.

A typical example showing the results of analyzing a sample of biochar by GC-MS to assess its purity. In this case, the compounds highlighted would be undesirable in the final product because of their known toxicity; work carried out at EBRI aims to understand the pyrolysis conditions that lead to these contaminants and minimize them. The analysis used a Frontier Lab EGA/PY-3030 double-shot analytical pyrolyzer, close-coupled with a GCMS-QP2010 SE system.

Bioliquids as fuel additives and antifungal agents

Bioliquids are also a fruitful area of study, says Dr. Chris Thomas, Knowledge Exchange Associate at EBRI and another former Ph.D. student of Dr. Nowakowski. *"A particular interest at the moment is what we call the 'catalytic upgrading' of pyrolysis liquids. This involves taking the crude, water-immiscible pyrolysis oil and treating it with hydrogen under high pressure and temperature with a catalyst. That removes oxygen from the oxygen-containing compounds, giving us an upgraded pyrolysis liquid, which can then be distilled and combined with conventional biorefinery streams for production of* ***biofuels."***

Dr. Thomas is keen to emphasize the benefits of this approach: *"Directly from what are often very complex chromatograms, we can use this 'simulated distillation' method to help us understand at what temperature the different components in a mixture will boil off at. The resulting distillation curve shows us the proportion of each component that evaporates at different temperatures, which is crucial for checking the quality of biofuels and other bioliquid-derived chemicals."*

Another application of bioliquids that Dr. Nowakowski and colleagues are working on is so-called ***"wood vinegar".***

Dr. Nowakowski explains: *"These liquids have received a lot of hype as 'natural' antifungals and antibacterials, but there's little information about exactly what they contain, let alone studies into their environmental and health impacts. So, a focus for us at EBRI over the coming year or so will be using our HPLC and GC-MS instruments to understand these liquids in more detail."*

This research is vital, he adds. *"Wood vinegar definitely has potential for treating mould and bacterial films, but there's a big issue around process and quality control. For example, if the pyrolysis temperatures are too high, the product can contain toxic aromatics, dioxins and chlorine-containing chemicals. If you're applying the wood vinegar to crops, they'll end up in the environment, which is something you certainly don't want."*

In summary, it seems that EBRI has a solution for every challenge: *"Whatever the feedstock type, we can select and tailor the process to extract the maximum value from it!"* he says.

Expanding biowaste research – with support from Shimadzu

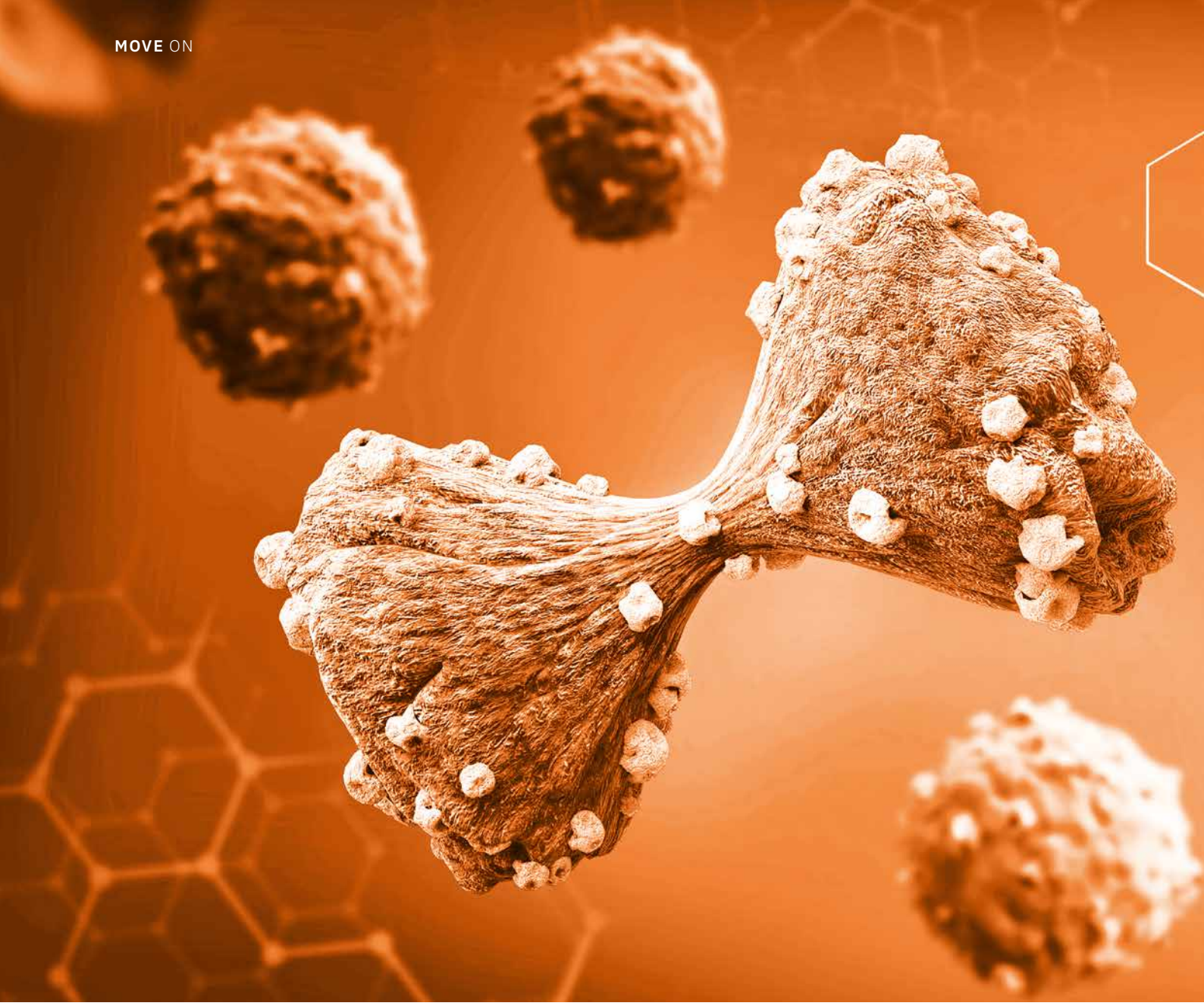
Through all of his work as a researcher and laboratory manager at EBRI, Shimadzu has been there for him, explains Dr. Nowakowski. *"Right from 2013 when we were setting up the new laboratories for bioenergy research here at EBRI, I've been able to benefit from their expertise in analytical chemistry,"* he states.

"They've always been ready to discuss our needs, and in many cases they've made a major contribution to the decision-making process. For example, in one project we needed to analyze permanent gases from our hydrothermal liquefaction unit, and they helped with setting up a GC-BID system – which significantly improved our research outputs compared to the GC-FID system we'd originally been planning."

This collaboration has continued to the present day, he adds, with guidance on getting the most out of the equipment as well as routine maintenance and timely support. *"With so many people booking our equipment, business continuity is very important for us – so it's great that we always have a quick response from Shimadzu!"* he says.

Note

For more information and references, please refer to the digital version of this edition.



Screening for DPD (dihydropyrimidine dehydrogenase) deficiency in patients needing fluoropyrimidine-based chemotherapy is becoming a strong recommendation – if not mandatory – by health authorities in Europe in order to reduce the risk of severe drug toxicities that these treatments can cause. Indirect phenotyping of DPD deficiency can be performed by measuring the level of uracil and dihydrouracil in plasma. To facilitate the detection of DPD deficiency, the DOSIURA™ reagent kit offers a turnkey analytical solution with all the needed reagents for the precise quantification of these compounds by LC-MS/MS.

Fluoropyrimidines, a group of anticancer drugs including 5-fluorouracil (5-FU), are widely used in the treatment of solid tumors such as colorectal, gastric, ENT and breast cancers. However, fluoropyrimidine-based chemotherapies could expose patients to severe side effects, occurring in one in five cases, and can cause severe toxicities (grades 3–4 according to the WHO classification of chemotherapy toxicities) and rarely but occasionally deaths (incidence between 0.1 and 1 %).[1]

DOSIURA™, a new solution for DPD deficiency screening

Uracilemia measurement by LC-MS/MS as an accurate approach

Sibylle Collard, Julia Petit, Mikael Levi, Alsachim

These toxicities may be linked to a deficiency in the activity of the main enzyme involved in 5-FU elimination, dihydropyrimidine dehydrogenase (DPD) (Figure 1).

DPD is an enzyme that assists the body in the catabolism of endogenous pyrimidines (uracil and thymine) and fluorinated pyrimidines such as 5-FU. With low activity or without enough DPD enzyme, these chemotherapy drugs accumulate in the body and cause more serious side effects than anticipated. Patients with a significant DPD deficiency receiving 5-FU treatment have an increased risk of toxicity. DPD activity varies from one individual to another, so it's essential to know whether a deficiency exists before starting treatment.

Today, many health regulatory authorities in Europe, and even worldwide, tend to recommend or require systematic DPD deficiency assessment in patients receiving fluoropyrimidine-based chemotherapy. →

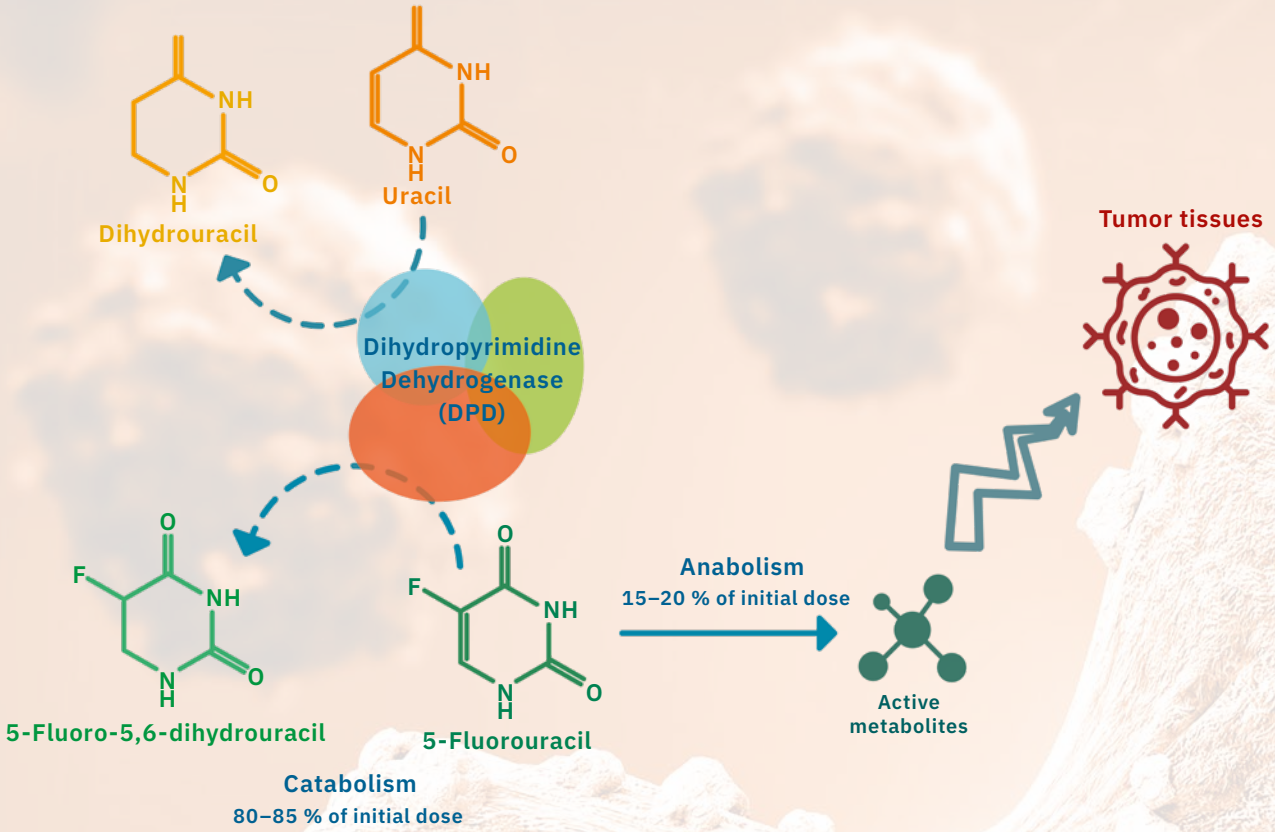


Figure 1: Scheme of DPD role in pyrimidine and 5-fluorouracil metabolism

Testing for DPD deficiency by genotyping or phenotyping

Genotyping is the search for variants of the DPYD gene encoding the DPD enzyme, while phenotyping involves direct or indirect measurement of the enzyme's activity.

Genotyping is currently based on the search for four variants: DPYD*2A, DPYD*13, c.2846A>T and HapB3. It can detect deficiencies but does not cover all populations,[1] and studies highlighted many toxicities not explained by mutation on these four variants.

The alternative method, phenotyping, involves direct measurement of DPD activity in PBMCs (peripheral blood mononuclear cells) or indirect measurement by quantification of uracil and dihydrouracil levels in plasma. However, direct measurement of the enzyme activity is very complex and time-consuming, thus, indirect phenotyping is preferred. Plasma concentrations of these two compounds are determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

DOSIURA™, a turnkey solution for rapid quantification of uracil and dihydrouracil

Alsachim, a Shimadzu Group company, has developed DOSIURA™, a turnkey solution for the quantification of uracil and dihydrouracil in plasma, enabling rapid assessment of DPD deficiency. This innovative reagent suite and its analytical method provide an answer to many of the problems faced by laboratories and hospitals in the phenotyping of DPD deficiency.

Following recommendations for preventive screening for DPD from health authorities like the European Medicines Evaluation Agency (EMEA) from 2020,[2] the ANSM in France and the MHRA in the UK, hospitals and biomedical laboratories are facing a double challenge: an increased demand for DPD deficiency analyses [3] and the need to develop long and highly complex sample preparation and analytical methods to perform these analyses.

DOSIURA™, the only one of its kind on the market, offers the required reagents and a rapid analytical method for DPD deficiency testing

Based on the indirect phenotyping approach, more reliable and reproducible, and the LC-MS/MS method offering simultaneous quantification of several compounds, the kit enables rapid measurement of uracil and dihydrouracil.

DOSIURA™ provides the quality reagents needed for these analyses: calibrants, internal standards (synthesized by Alsachim's expert chemists) and controls, eliminating the complexity and analytical instability associated with reagent preparation for laboratories. *"The controls are prepared in real human plasma and formulated at decision threshold concentrations for DPD deficiency assessment, which facilitates the clinician decision-making process following analysis of the results,"* explains Julia Petit, project leader in R&D at Alsachim.

Uracil and dihydrouracil are polar endogenous compounds with low molecular weights, making method development a real challenge for the R&D team, especially the separation of target compounds from all interfering molecules present in the matrix. *"Our objective was to provide our customers with a fast, accurate and robust solution,"* says Julia Petit.

The DOSIURA™ solution offers metrological traceability of these compounds and a very fast sample preparation method: the protocol and the reagents provided allow faster sample preparation compared to published methods.[4, 5] *„We aimed to offer a complete and rapid solution to tackle the problems that arise in hospitals and laboratories. Screening for DPD deficiency is a major issue, and through DOSIURA™ we are willing to support research into the treatment of cancer patients and to be a key partner for our customers in their daily work."*

Ensuring the safety of cancer treatment

With DPD deficiency screening becoming more widespread in countries around the world and with the entry into force of the IVDR and its requirements in terms of medical devices and standardization of methods, DOSIURA™ can be a major asset for standardizing and routinely analyzing uracil and dihydrouracil concentrations in a rapid fashion.

DOSIURA™ is for research use only, not provided to diagnostic use.

Note
For more information and references, please refer to the digital version of this edition.

Figure 2: DOSIURA™ turnkey solution for LC-MS DPD deficiency check in plasma

Pure to green

The role of water quality in power-to-gas applications

Markus Janssen, Shimadzu Europa GmbH

Green hydrogen could turn the dream of a sustainable supply of energy and raw materials into a reality. But how is this gas actually produced from renewable energy? And what role does water quality play in this? The following article has the answers. It addresses key technologies and innovative devices that not only ensure water quality but also contribute significantly to the efficiency and service life of electrolysis plants, which are indispensable for hydrogen production.

"My friends, I believe that water will one day be employed as fuel, that hydrogen and oxygen, which constitute it, will furnish an inexhaustible source of heat and light of an intensity of which coal is not capable. [...] Water will be the coal of the future." These are the words of Jules Verne in his famous novel "The Mysterious Island". Words that laid the foundation for science fiction literature in 1874 and are becoming a reality today. And it almost sounds too good to be true: Hydrogen is neither toxic nor radioactive, it burns without producing pollutants and is also the most abundant element in the universe. In principle, it is technically possible to use hydrogen in many areas, such as industry, transportation and for heating. From an energy efficiency perspective, however, this does not always make sense, since using renewable electricity directly in many cases yields greater savings in greenhouse gas emissions.[1] But in cases where electrification is technically impossible or uneconomical, the use of hydrogen is a sustainable alternative.

From energy to gas

However, it's not quite that simple because molecular hydrogen (H_2) practically does not occur on earth in the form of a pure gas but predominantly in bound form and as a component of water. This means it cannot simply be obtained as a primary energy source like natural gas, for example, but must be produced using other energies. The energy source used for this then determines how sustainable the hydrogen economy itself becomes. Currently, H_2 is mainly produced by steam reforming from the methane contained in natural gas and biogas; this is referred to as gray hydrogen. However, this also releases an average of 10 tons of CO_2 into the atmosphere per ton of hydrogen produced.[2] If it is, on the other hand, produced exclusively using renewable energies in the so-called "power-to-gas process" (P2G), then it is referred to as green hydrogen. But how is energy converted into gas?

The first step in the power-to-gas process is generating electricity based on renewable energies as the primary energy source. This electrical energy is then converted into chemical energy by means of electrolysis. In the case of hydrogen, the technology has been well established for a long time and is often even demonstrated as a practical experiment during school lessons. Two electrodes are immersed in a water bath and connected to a direct current source. With sufficient voltage, the electric current breaks the water down into its components hydrogen and oxygen. →

A “stack” of key technologies

The school experiment, which is often carried out using lye as the electrolyte, is very similar to the principle of alkaline electrolysis (AEL), which has been used for decades to generate H_2 on an industrial scale. There are disadvantages to this process, however, which may make it less suitable for the production of green hydrogen. Fluctuating power sources, such as wind power or photovoltaics, require a cyclical start-up and shut-down of hydrogen production and at least partial-load operation. AEL is less suitable for this, as it requires long start-up times and there can be problems with gas quality in partial-load operation.[3] By contrast, “PEM electrolysis” is considered a key technology for the production of green hydrogen and thus for the energy transition.

A proton exchange membrane (PEM) consists of a thin, solid polymer material such as Nafion, which can conduct protons (H+ ions) but is impermeable to electrons and gases. In a PEM electrolyzer, this membrane is the key component of every cell and is located between the anode and cathode, which are often coated with a catalyst to accelerate the electrolytic reactions. A central element of a PEM electrolyzer is the so-called “stack”, which consists of several cells stacked on top of each other – hence the name (Figure 1). Bipolar plates serve as current conductors between individual cells and dissipate the gases produced. PEM electrolysis is a high-pressure process that enables high current densities. This high current density leads to more efficient hydrogen production, as more hydrogen is produced per unit of time. This makes PEM electrolysis a preferred technology when it comes to producing green hydrogen because, unlike AEL electrolysis, it can be started up more quickly and has short reaction times with fluctuating electricity production.

Efficiency through purity

The purity of the feed water is crucial for the efficiency and service life of PEM electrolyzers. In stoichiometric terms, nine liters of water are needed to produce one kilogram of green hydrogen. However, this water must meet certain quality standards in order to avoid stack failures and reduced performance. Impurities, such as dissolved salts, minerals and organic compounds, can cause irreversible damage [4], impair the performance and service life of the membranes and can negatively affect hydrogen quality. For this reason, strict specifications for electrical conductivity and total organic carbon (TOC) content must be met, often based on international guidelines for ultrapure water (Table 1). It’s vital that these parameters are continuously monitored for the long-term and economical production of green hydrogen.

Hydrogen electrolysis plants are often built near solar and wind farms. However, there may be a lack of sufficient fresh water in sunny, dry areas, while in windy coastal regions the available water is usually saline. Such conditions require special water treatment technologies (Table 2) to make the available raw water usable for PEM electrolysis. →

Contaminant group	Possible negative impact	Measuring parameters	Example of limit value
Organic compounds	Membrane fouling, corrosion, biofilm, hydrogen impurity	TOC	< 50 ppb
Ions/inorganic impurities	Conductivity reduction, catalyst damage, H_2 impurity, corrosion	Electrical conductivity Spec. resistance	< 0.1 µS/cm > 10 MΩcm

Table 1: Example of a water specification for a PEM electrolysis stack

Processing technology	Short name	Removed contaminants
Sand filtration/aeration	Prefiltration	Iron and manganese
Ultrafiltration	UF	Particles, organic matter, microbiology
Softener/antiscalant dosage	Softener	Hardness-causing ions
Membrane degassing		Dissolved gases (e.g. CO_2)
Reverse osmosis	RO	Salt, particles, microbiology, ionic load, organic matter
Electrodeionization	EDI	Removal of ions and ionizable contaminants
Ion-exchange resin	Polisher	Final contaminants

Table 2: Rough overview of treatment techniques for electrolysis

Figure 1: PEM electrolyzer

Ensuring water quality using PAT

Monitoring and securing water quality in an electrolysis plant can be significantly improved by using process analytical technology (PAT). In addition to the water pretreatment system, the ultrapure water loop, which is used for both water supply and heat dissipation, plays a crucial role in guaranteeing trouble-free operation and avoiding additional costs. Impurities can enter the loop during operation, which is why a partial flow is purified using an ion exchange resin before being fed into the anode. An online TOC analyzer can be used at this point for final quality control using PAT. Since there is no direct correlation between the TOC and electrical conductivity parameters and the presence of contaminants, both parameters should be continuously measured. Analyzers such as the Shimadzu TOC-1000e (Figure 2) provide both readings at short time intervals, allowing early detection of contamination and enabling predictive maintenance of the electrolyzer.

The TOC-1000e is specifically designed for online monitoring of ultrapure water and provides precise measurement of both TOC content with a detection limit of 0.1 µg/L and the electrical conductivity of water. Its innovative technology includes a mercury-free excimer lamp that generates ultraviolet light at 172 nm to break down even difficult-to-oxidize components, ensuring that no contaminant goes undetected. The lamp is only switched on when needed which extends its service life and doubles the usual operating life of the device to one year compared to conventional instruments. This means that the TOC-1000e can operate automatically for up to a year. Another highlight is the so-called “Active Path”, in which the sample flows directly through the lamp to ensure the most efficient irradiation possible, minimizing contamination and carry-over effects. On top of this, the TOC-1000e enables annual calibration and maintenance quickly on site, which means that the analyzer does not have to be sent in. It stands out for its excellent connectivity, offering bidirectional bus communication and a built-in web server for easy remote diagnostics and a detailed view of data, including history. Despite its extensive range of functions, the TOC-1000e is small and light, weighs less than 3 kg and has a front panel the size of an A4 sheet of paper.

Its mobility allows it to be used for maintenance and troubleshooting on other parts of the water treatment system in the electrolysis plant.

Clean water for green hydrogen

Green hydrogen is at the heart of efforts to achieve a sustainable energy supply. Although there are challenges, especially when it comes to ensuring water quality and adapting to different geographical conditions, the technical advances offer great hope. Key tools such as process analytical technology (PAT) and instruments such as the TOC-1000e are crucial for monitoring and ensuring water quality. As the transition to a hydrogen economy moves forward, constant innovation and optimization is needed to increase the efficiency and sustainability of hydrogen production. In this way, green hydrogen has the potential to play a central role in our sustainable energy and raw material supply.

Note
For more information and references, please refer to the digital version of this edition.

Figure 2: Shimadzu TOC-1000e

analytica
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