Recent developments in stable isotope research: Joint European Stable Isotope User Meeting 2008

Joint European Stable Isotope User Meeting 2008
31st August–5th September 2008, Giens, France

A rapidly increasing number of applications are currently emerging from stable isotope research whilst, at the same time, significant evolution of the instrumentation may also be observed. The European stable isotope community gathered in Giens, France, to exchange views and discuss recent developments.

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The analysis of stable isotopes is becoming increasingly important in a variety of scientific disciplines. Originally introduced by geoscientists, the number of biological, biomedical and forensic applications has been increasing dramatically within the last few decades. However, depending on the peculiarities of a given application, **stable isotope analysis** (SIA) may represent a major challenge to the analyst. Very often, development and improvement of the methodology will turn out to be much more difficult to address than the actual scientific question. This is certainly one of the reasons why stable isotope researchers tend to meet at multidisciplinary meetings and exchange experience, strategies and solutions across the boundaries of scientific domains.

The second Joint European Stable Isotope Users Meeting (JESIUM 2008 [101]) was organized in this spirit, and featured a broad coverage of stable isotope methodologies and scientific approaches. However, the vast majority of the presented applications employed light elements, giving rise to possible interest to the bioanalyst. In this context, only a few projects still rely on classically labeled material. By contrast, the exploitation of very small variations in stable isotope ratios has grown into a standard methodology. Either the signals present in natural changes of stable isotope ratios are measured or low enrichment **labeling** experiments are performed. This tendency is due to the increasing availability of **isotope ratio mass spectrometry** (IRMS) coupled on-line to powerful separation technologies, such as gas chromatography (GC) and liquid chromatography (LC).

This report focuses on these techniques and their applications, as these typically have considerable bioanalytical relevance. Nonetheless, it has to be mentioned that significant progress also occured within neighbor methodologies. As an example, the documented progress in accuracy and precision of tunable laser diode spectroscopy was most impressive. Foremost field work concerning isotope analysis of gases was presented, but in the future, developments that are also beneficial to the biosciences may be expected. Similarly, IRMS based on inductively coupled plasma ion sources (ICP-IRMS), for the most part, still appears to be restricted to geoscientific applications.

LC–IRMS

LC–IRMS is currently restricted to the analysis of stable carbon isotope ratios ($^{13}C/^{12}C$) (see [1] for a recent review of the technology). The mandatory complete postcolumn conversion of the analytes to carbon dioxide precludes the presence of organic compounds in the mobile phase. This, of course, further restricts the number of possible applications. Nonetheless, for numerous reasons there is significant interest in SIA of amino acids (AAs) and carbohydrates (CHs).

In contrast to other mass spectrometric detection systems, a prerequisite for valid results from LC–IRMS is outstanding chromatographic resolution. Virtually complete baseline separation of adjacent peaks is required in order to obtain accurate and precise isotope ratios. However, the fulfillment of this prerequisite seems to be less problematic compared with GC–IRMS where, in addition, it is desirable to perform the analyses on underivatized compounds as additional carbon will blur the original isotopic signature.

Baseline separation of the majority of underivtized biological AAs was achieved by McCullagh *et al.* (Oxford, UK; Langenargen & Hohenheim, Germany). They employed an acidic mixed-mode high-performance LC column with a gradient of sulfuric acid in the

STABLE ISOTOPE ANALYSIS
Quantitative analysis of stable isotopes. Typically ratios of isotopes are calculated

LABELING
Introduction of isotopically enriched material in order to track metabolism or flow

ISOTOPE RATIO MASS SPECTROMETRY
Simple but highly accurate and precise mass spectroscopy technology designed to detect subtle differences in isotope ratios

Future science part of fsg

mobile phase. The background of the study was physiological, but the talk focused on methodological aspects, as did many presentations based on the relatively new concept of LC–IRMS. As was demonstrated by Abaye *et al.* (East Kilbride, Scotland), comparably good results can be achievable with the use of anion exchange columns. An interesting application of LC–IRMS was presented by Fuller *et al.* (Leipzig, Germany). The group was able to trace the carbon flow of specific amino acids from human breast milk to the fingernails of infants. The authors intend to provide a better understanding of trophic level effects in the human. The background of this work is archaeological, but these data will certainly be of interest to pediatrists and nutritional scientists.

Again, focusing on the demand for underivatized analytes and optimization of the separation, Moerdijk-Poortvliet (Yerseke, The Netherlands) presented an LC–IRMS method for a variety of CHs. It is based on strong anion exchange columns and employs NaOH as the eluent. Analysis of acidic CHs was facilitated by addition of nitrate to the mobile phase. Likewise, Bode *et al.* (Ghent University, Belgium) employed anion exchange for the LC–IRMS analysis of CHs, specifically amino sugars and acidic amino sugars. Two separate isocratic systems based on identical columns were employed. Both CHs applications have an ecological background. For general purpose applications, Godin *et al.* (Nestle Research, Lausanne, Switzerland) demonstrated rapid and precise SIA of underivatized glucose by LC–IRMS. This certainly has potential for a variety of clinical and physiological questions.

The synthesis rate of glutathione in preterm neonates was investigated by Schierbeek *et al.* (Rotterdam, The Netherlands). The group performed simultaneous LC–IRMS analysis of glutathione dimers and of the precursor glycine following infusion of the ^{13}C-labeled amino acid. Only 100 µl of blood was required. This impressively demonstrates the potential of SIA to perform meaningful studies based on very small sample sizes and thus, to significantly extend the possible set of matrices and subjects.

Generally, the number of successful LC–IRMS applications seems to be steadily increasing. The restriction to completely inorganic mobile phases certainly discouraged possible users immediately after the introduction of this technique, but in the meantime a number of creative and promising approaches have emerged. Once these have matured, LC–IRMS will undoubtedly become a powerful standard tool for a growing community.

Natural abundance
Stable isotope research relying on natural variations of stable isotope ratios

Isotope signature
Isotope ratio characteristically resulting from specific sources or processes

GC–IRMS

While LC–IRMS technology is categorically restricted to the analysis of carbon isotopes, GC–IRMS is capable of measuring hydrogen, carbon, nitrogen and oxygen isotopes [2]. However, typically, the hardware setup has to be slightly modified when a different element is to be investigated. By contrast, the hyphenation of elemental analysis and continuous-flow IRMS may yield stable isotope ratios simultaneously for more than one element. This is of increasing interest, as isotope patterns often bare much more information than the signature of a single element. Thermo Fisher Scientific (Bremen, Germany) introduced a GC–IRMS interface capable of immediate change from oxidative (C, N) to reductive conversion (H, O). Nonetheless, different elements cannot yet be analyzed within a single run. Much tedious work may yet be dropped.

As is well known, and has been outlined previously, LC techniques are the method of choice when it comes to the analysis of AAs. However, studying nitrogen stable isotopes in AAs very often yields more information than carbon isotopes. Consequently, GC–IRMS has to be employed, and this of course requires derivatization of functional groups. After conversion to *N*-pivaloyl-i-propyl esters, Klaus Petzge (Potsdam, Germany) performed GC–IRMS of AAs derived from human hair. The study was carried out in order to find to what degree dietary composition is reflected in the stable isotope ratios of these compounds and what influence dietary changes might have.

Likewise, Tea *et al.* (Nantes, France) investigated nitrogen isotopes by GC–IRMS. They were interested in taurine in order to detect administration of very small amounts of the labeled compound to low-weight neonates for diagnostic purposes. Dual derivatization to the imidic acid ethyl ester/sulfonic acid ethyl ester was performed beforehand.

There is increasing interest in the **natural abundance** of carbon isotopes of steroid hormones, mainly because these have an enormous potential for abuse and because the synthetic compounds might betray their presence by the stable carbon **isotope signature**. Thomas Piper *et al.* (Cologne, Germany) presented results from a single dose administration of deyhdroepiandrosterone. Based on the GC–IRMS

analysis of a multitude of possible metabolites, new insights into the metabolism of this prohormone could be obtained. The residence time of the metabolites varies significantly. Consequently, they are unequally suited as possible target compounds. The method employs acetylation of the analytes and thus, requires some correction for the additional carbon.

A more introductory presentation into the topic was given by Christope Saudan (Lausanne, Switzerland). His group also demonstrated that there are characteristic differences in the isotope signatures of urinary steroids in different human populations. This can be attributed to varying composition of the diet.

Some fundamental research was performed by Flenker *et al.* (Cologne, Germany) who investigated the kinetics of the propagation of dietary isotope signatures to urinary steroids. In particular, the role of cholesterol in the diet, the biochemical steroid precursor, was investigated. Significantly less cholesterol is converted to steroid hormones than would be expected.

Steroid abuse is also an issue in stock breeding, at least in the European Union, where these compounds are systematically prohibited. However, the methodology is even more complicated for humans, because more purification steps are required in order to obtain uncontaminated isotopic signals. Emanuele Bichon and colleagues (Nantes, France) presented detailed considerations concerning the establishment of natural abundance isotopic threshold values for cattle. Interestingly, the criteria established in humans for doping control purposes also seem to be applicable to veterinary forensics. In another study, the same group introduced the detection of the administration of synthetic cortisol to cattle by carbon SIA.

Other techniques

One of the classic bioanalytical methodologies based on SIA has been the analysis of breath gases following the administration of labeled material, either for the purpose of diagnostic testing or in order to study metabolism and its kinetics. Using IRMS to analyze breath following administration of a dual ^{13}C-label, Wutzke *et al.* (Children's Hospital, University of Rostock, Germany) deduced a shortened oreocaecal transit time following administration of L-carnitine.

Since the early days of stable isotope analysis, paleontologists and archaeologists have tried to exploit the isotope signatures preserved in bulk hair, teeth and bones. Often the diet of fossil organisms, including the human, is intended to be reconstructed. Julia Lee-Thorpe's (Bradford, UK) keynote lecture stressed the difficulties in the interpretation of such data; as did Luca Lai (University of South Florida, USA), while suggesting several environmental correction factors.

Frank Hülsemann (Cologne, Germany) demonstrated characteristic changes of the nitrogen isotope signature in bulk hair during increased workload. In an impressive case study, Hülsemann presented data from a 600 km walk across the world's most arid desert, the Atacama (Coquimbo, Chile), inducing a loss of 10 kg body mass in the subject. Apart from athletic merits, this study is hoped to yield substantial knowledge to support the interpretation of ancient hair samples and to develop diagnostic tools for sports and nutritional scientists.

Conclusions

SIA is increasingly being seen as a standard tool for answering very diverse scientific questions. It has become essential to forensic bioanalytics, but clinical investigations now increasingly rely on stable isotopes because they are felt to represent close to ideal tracers. Often, synthetic labeling is not required because variable natural abundances of stable isotopes can be exploited. The sensitivities of modern IRMS allows for the detection of incredibly small changes of isotope abundances. These circumstances, as well as the harmlessness of stable isotopes, vastly extend the possibilities of tracing metabolism in the broadest sense, that's to say from cellular to ecological scales. These progresses have mostly been facilitated by recent improvements in the instrumentation, particularly with the introduction of GC–IRMS and LC–IRMS interfaces. By contrast, SIA also confronts the analyst with new challenges. As an illustrative example, most well-established LC methods cannot even approximately be transferred to LC–IRMS. Sample preparation procedures also tend to become much more demanding.

These facts were well reflected at the JESIUM in 2008. However, an increasing number of groups bare the challenges of SIA and provide new and interesting applications. Currently, methods are rapidly being developed for more isotope systems, compounds and classes of compounds. Concurrently, the development of the instrumentation is far from complete. While the principle design of IRMS has not been changed since Nier's stroke of genius in 1940 [3], the

improvement of peripherals is still an important engineering playground. Well documented by JESIUM, innovative developments in isotope analysis are emerging at a continually accelerating rate. This applies to fundamental research, as well as to the establishment of standard applications.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

1 Godin JP, Fay LB, Hopfgartner G. Liquid chromatography combined with mass spectrometry for ^{13}C isotopic analysis in life science research. *Mass Spectrom. Rev.* 26(6), 751–774 (2007).

2 Sessions AL. Isotope-ratio detection for gas chromatography. *J. Sep. Sci.* 29(12), 1946–1961 (2006).

3 De Laeter J, Kurz MD. Alfred Nier and the sector field mass spectrometer. *J. Mass Spectrom.* 41(7), 847–854 (2006).

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101 *JESIUM 2008.* Giens, France, 31 August–5 September 2008 (Accessed 28 November 2008) www.jesium2008.eu