National Measurement System

Light Isotope Ratio Measurements: Applications Food Authenticity/Origin Determination

Philip J H Dunn, Dmitry Malinovsky and Heidi Goenaga-Infante

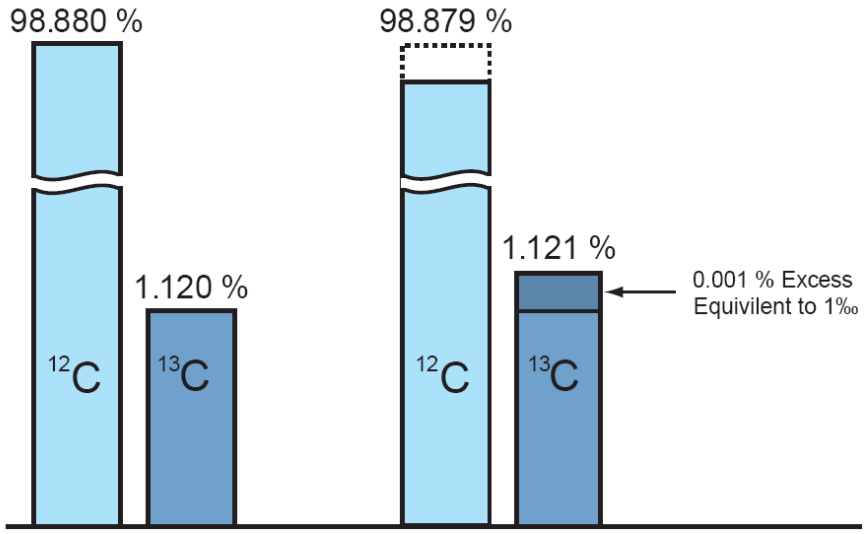
Science
for a safer world

Outline

- Stable isotopes and variations in abundances/ratios.
  - Focus on carbon.
  - Applications in food authenticity and origin discrimination
- How do we measure isotope ratios using EA-IRMS?
- Traceability and the characterisation of new matrix-matched reference materials
  - Absolute isotope ratio measurements and a new approach to reference materials.
- Conclusions & future work.

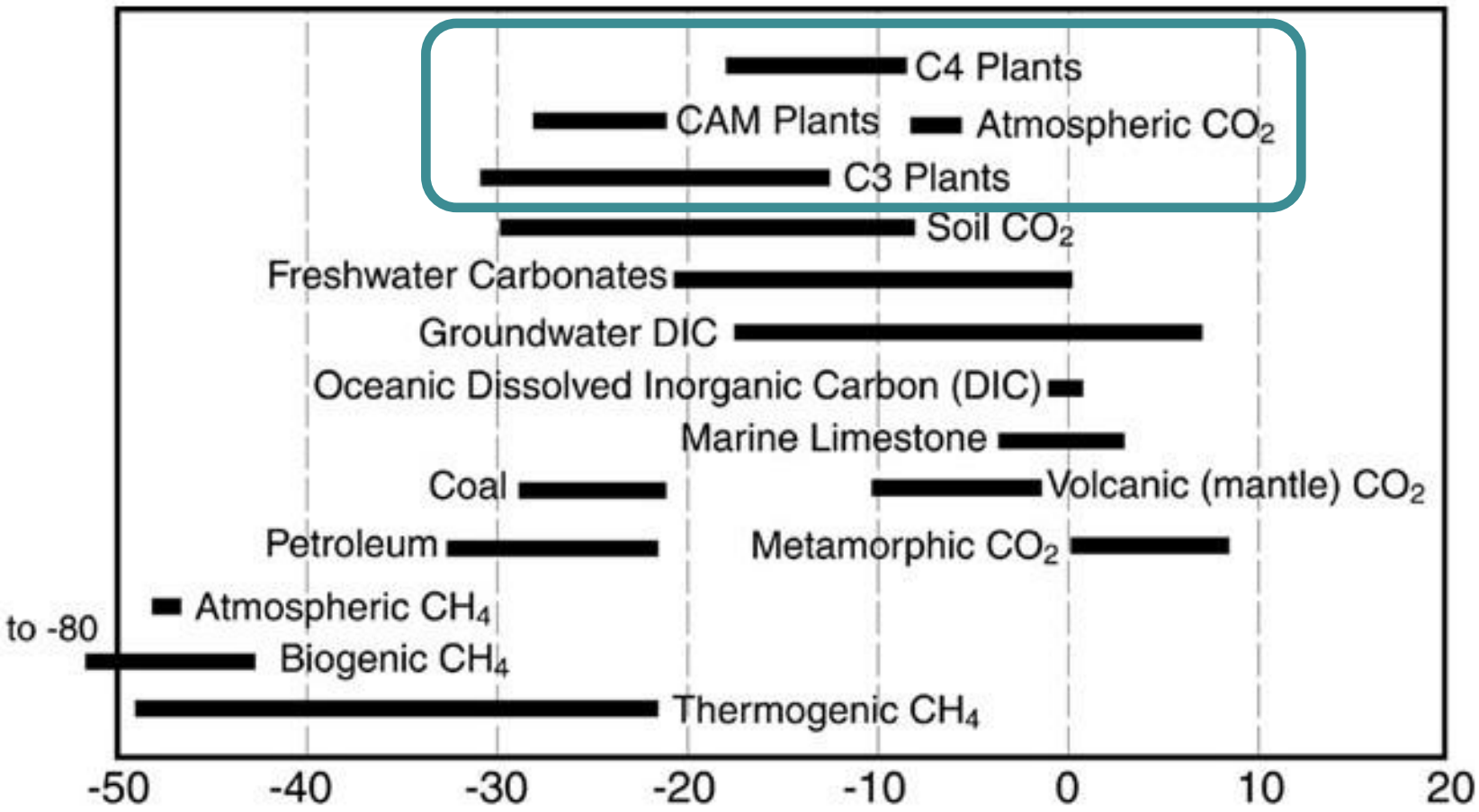
Carbon Isotope Ratios

- Carbon has two stable isotopes: ^{12}C (~99%) and ^{13}C (~1%).
- There can be subtle variations in the absolute ratio of $^{13}C/^{12}C$.
- These are commonly expressed as delta values relative to a standard.
- Delta values small so multiplied by 1000.



$$\delta^{13}C = \left(\frac{^{13}R_{sample}}{^{13}R_{Std}} - 1 \right)$$

Natural C isotope ratio variations



C isotopes for Food Authenticity/Origin

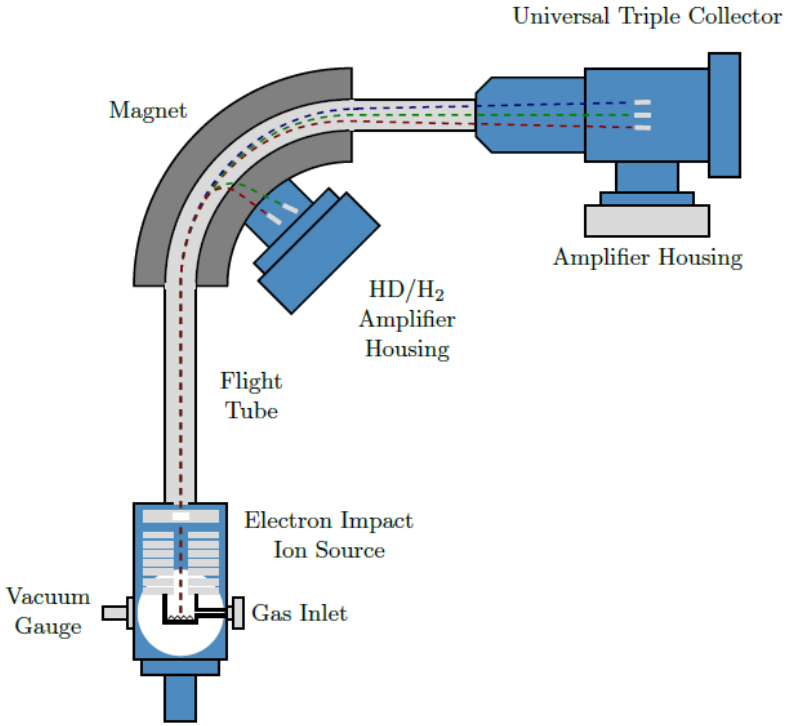
- C3 vs C4:
  - Cane vs Beet sugar
  - High fructose corn syrup adulteration in Honey
  - Corn fed chicken
  - Added sugar in wine
- Multi-element approaches (C with H, N, O, S) for protected origin foods:
  - Scotch Beef, Welsh Lamb, Scotch Whiskey, olive oil, balsamic vinegar, cheeses, fruit juices etc
  - H and O isotopes in particular for geographical origin

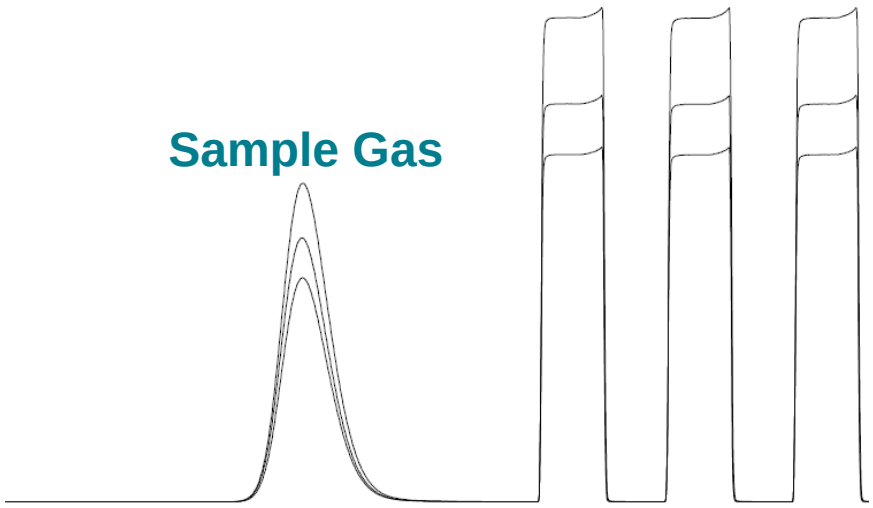
EA-IRMS measurement

Material into tin capsules

Autosampler → Combustion/ Reduction Reactor → Water Trap → Separation Column → Sample Open Split → IRMS

"Reference" Gas → Reference Open Split → IRMS





Calibration/Normalisation

- Reference materials of known/certified $\delta^{13}C$ value analysed in same sequence as unknown samples:
  - **Identical treatment** of sample and RM.
  - Calibration plot to normalise results to international scale.
  - Limited numbers of RMs available (NIST, IAEA, USGS).
- Reference materials therefore **critical** for high quality IRMS data.
- **Traceability** to international scales is essential for inter-laboratory comparability.

Carbon Isotope Standards & Scales

- Harmon Craig (1957)
  - Peedee Belemnite – calcium carbonate from South Carolina used by Urey's group in Chicago as zero point.
- PDB ran out so a new carbon isotope scale known as VPDB was introduced:
  - Defined by assigning $\delta^{13}C_{VPDB}$ of NBS-19 = +1.95 ‰ (exact).
- VPDB now also defined by $\delta^{13}C_{VPDB}$ of LSVEC = -46.6 ‰ (exact) for scale expansion (VPDB-LSVEC).
- Absolute isotope ratio of VPDB determined a number of times with little agreement

Traceability

- Scales traceable to an artefact.
  - Can develop independently (e.g. oxygen delta scales).
  - Need to maintain the scale (drift in reference artefacts, difficult/impossible to regenerate).
  - Provide very consistent results.

- Scales traceable the SI (i.e. a measurement process).
  - Provide accurate results.
  - Can have more than one source.
  - Enhanced metrological traceability

Chemical and Biological Metrology LGC Project

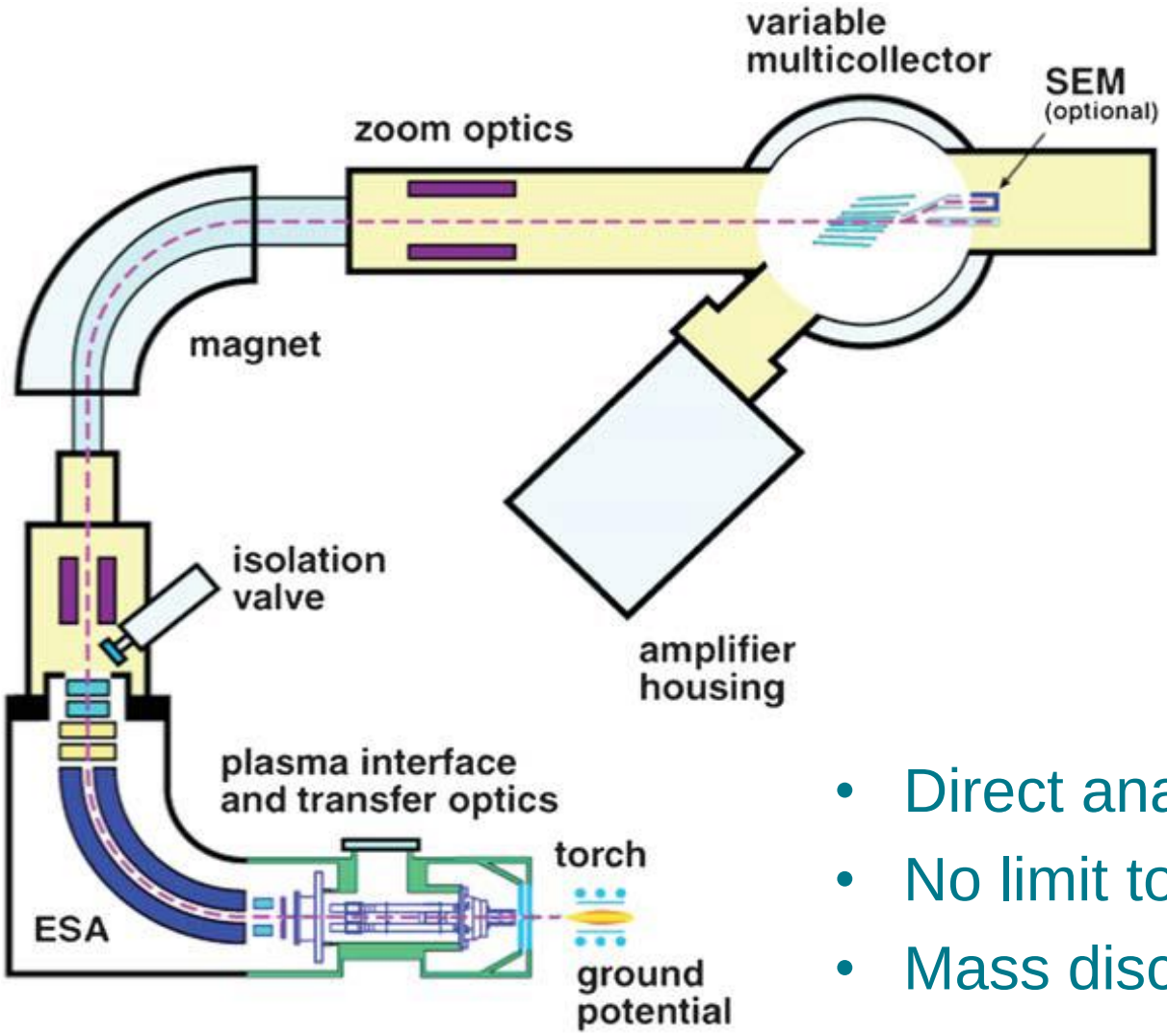
National Measurement System

LGC

- Certify a new reference material for absolute C isotope ratio which is traceable to the SI (kg)
- Certification by MC-ICP-MS
- Confirmatory measurements by EA-IRMS

- Material useful to food analysis/authentication
  - Glycine
  - ^{13}C depleted isotopic composition
  - Amenable to EA- and LC-IRMS (GC-C-IRMS would require derivatization)

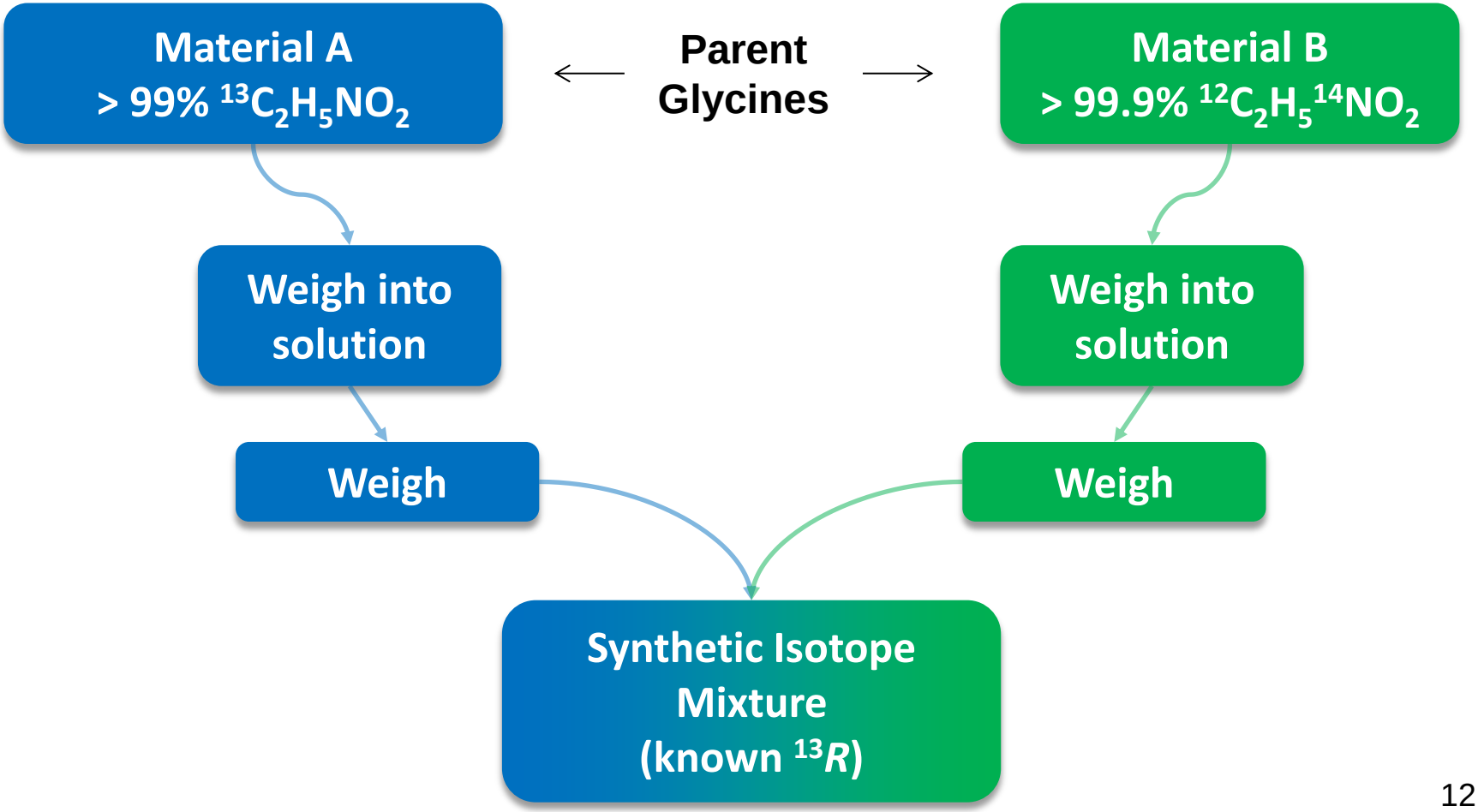
MC-ICP-MS

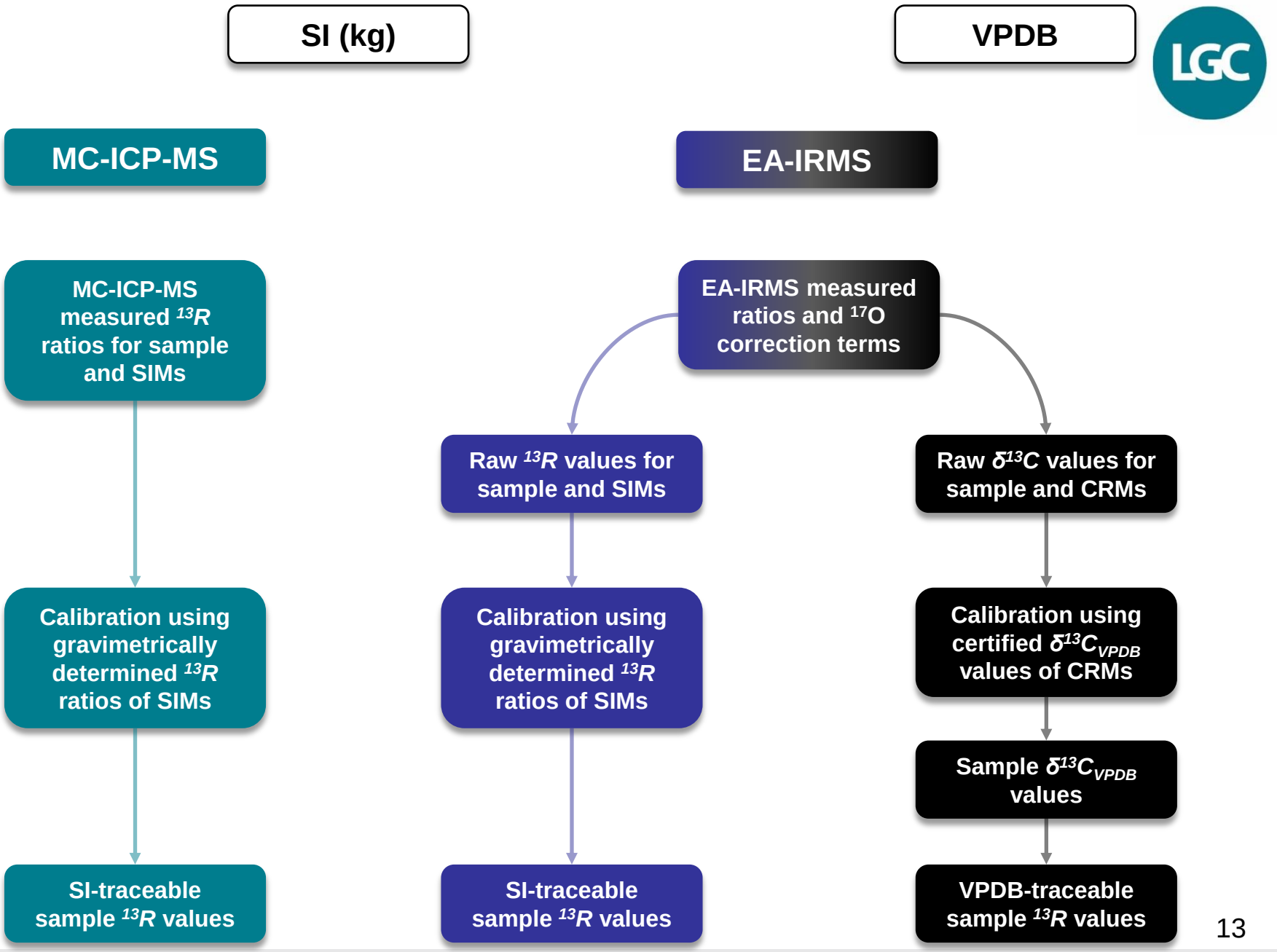


- Direct analysis of $^{12}C^{+}$ and $^{13}C^{+}$
- No limit to isotope ratio range.
- Mass discrimination.

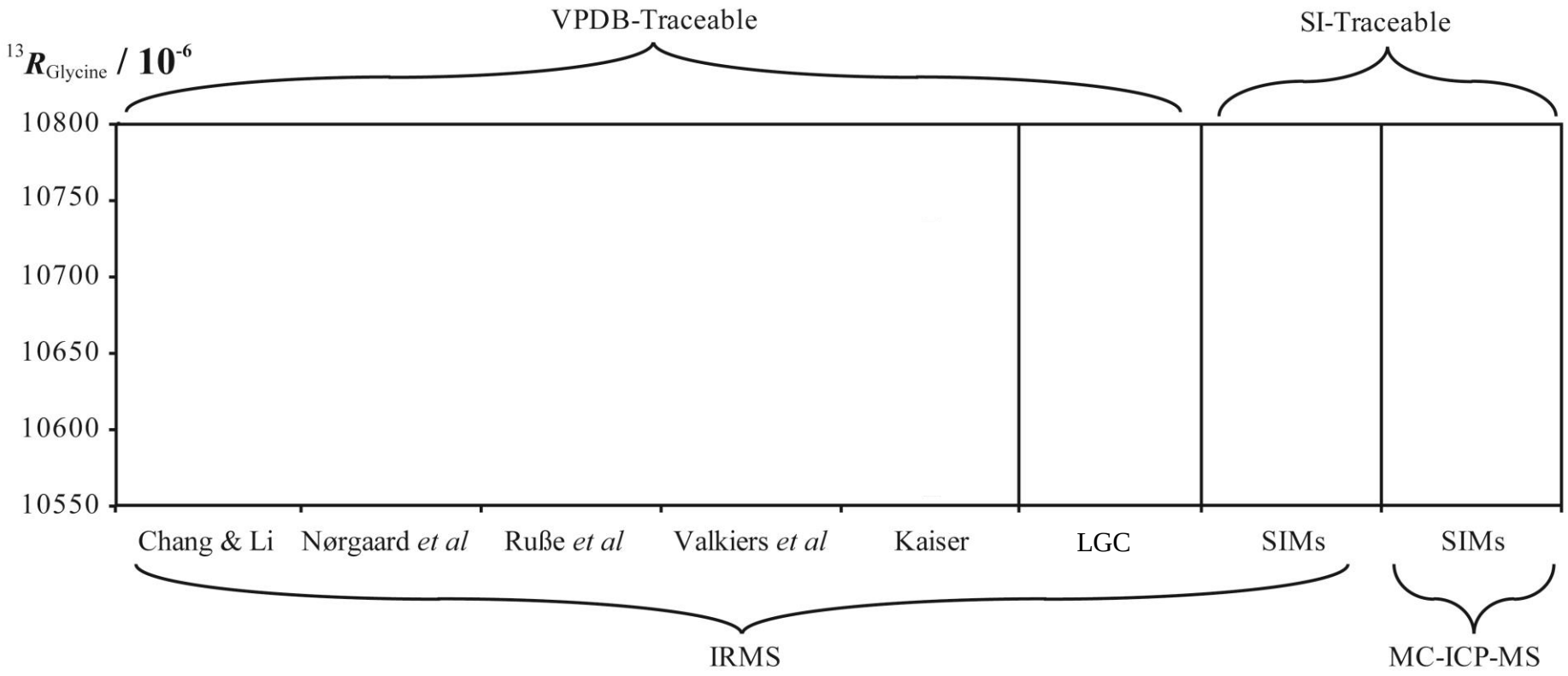
Jakubowski et al, J. Anal. At. Spectrom, 2011, 26, 693-726

Synthetic Isotope Mixtures – calibration to the SI




SI (kg)
VPDB
MC-ICP-MS
EA-IRMS
MC-ICP-MS measured ^{13}R ratios for sample and SIMs
Calibration using gravimetrically determined ^{13}R ratios of SIMs
SI-traceable sample ^{13}R values
EA-IRMS measured ratios and ^{17}O correction terms
Raw ^{13}R values for sample and SIMs
Calibration using gravimetrically determined ^{13}R ratios of SIMs
SI-traceable sample ^{13}R values
Raw $\delta^{13}C$ values for sample and CRMs
Calibration using certified $\delta^{13}C_{VPDB}$ values of CRMs
Sample $\delta^{13}C_{VPDB}$ values
VPDB-traceable sample ^{13}R values

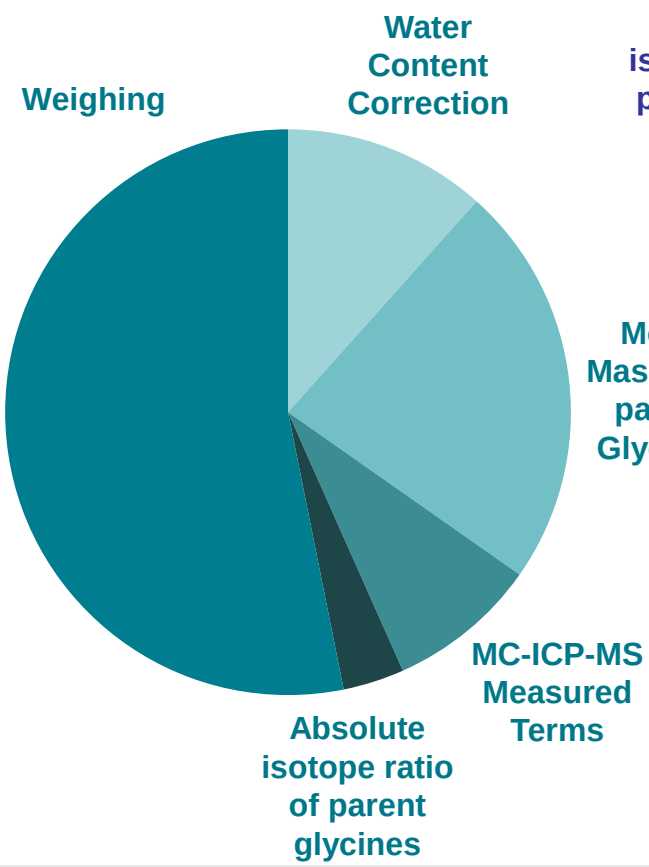
Results



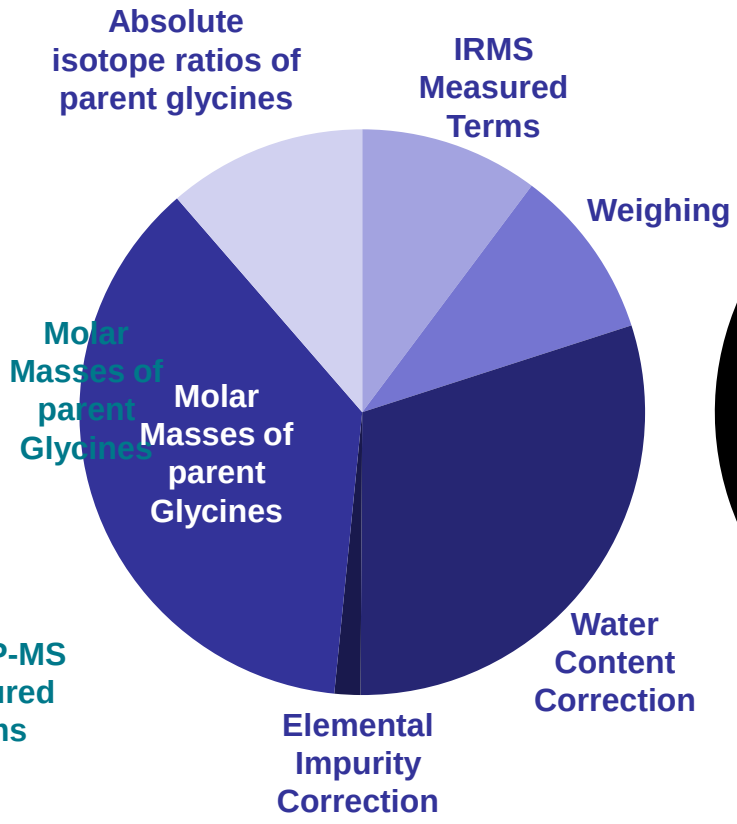
Dunn et al, Anal Bioanal Chem, DOI 10.1007/s00216-014-7926-1

Measurement Uncertainty

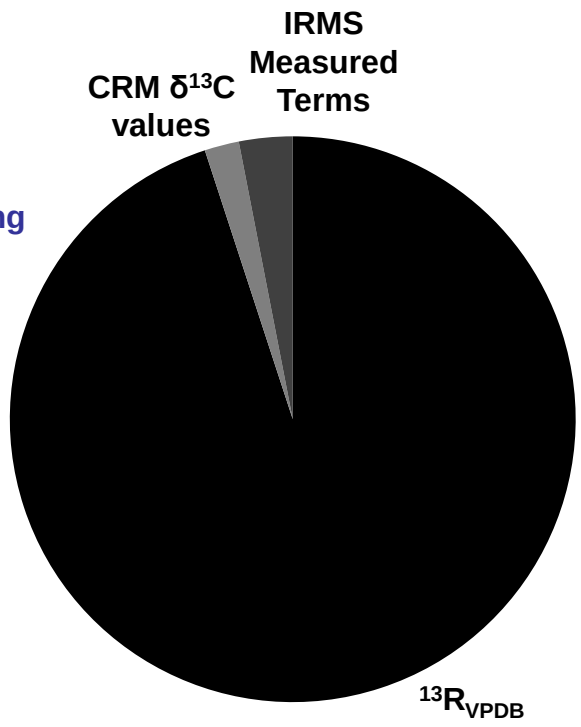
MC-ICP-MS via SIMs



IRMS via SIMs



IRMS via VPDB



Publications

Anal Bioanal Chem
DOI 10.1007/s00216-014-7926-1

RESEARCH PAPER

Calibration strategies for the determination of stable carbon absolute isotope ratios in a glycine candidate reference material by elemental analyser-isotope ratio mass spectrometry

Philip J. H. Dunn · Dmitry Malinovsky · Heidi Goenaga-Infante

Received: 20 March 2014 / Revised: 22 May 2014 / Accepted: 23 May 2

Abstract We report a methodology for the determination of the stable carbon absolute isotope ratio of a glycine candidate reference material with natural carbon isotopic composition using EA-IRMS. For the first time, stable carbon absolute isotope ratios have been reported using continuous flow rather than dual inlet isotope ratio mass spectrometry. Also for the first time, a calibration strategy based on the use of synthetic mixtures gravimetrically prepared from well characterised, highly ^{13}C-enriched and ^{13}C-depleted glycines was developed for EA-IRMS calibration and generation of absolute carbon isotope ratio values traceable to the SI through calibration standards of known purity. A second calibration strategy

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Cite this: *J. Anal. At. Spectrom.*, 2013, **28**, 1760

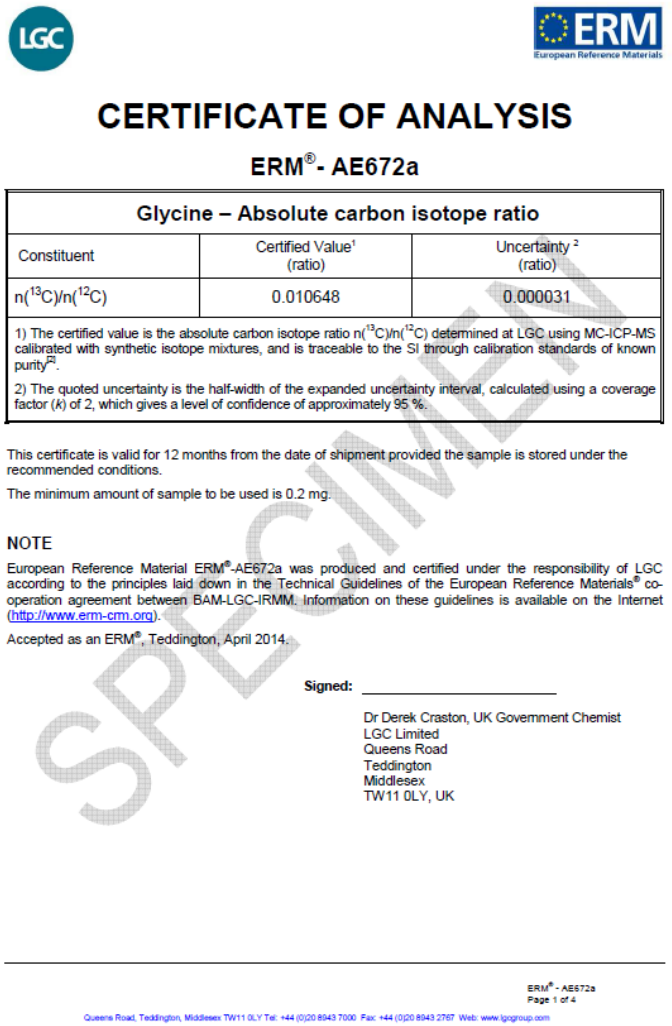
Determination of absolute $^{13}C/^{12}C$ isotope amount ratios by MC-ICPMS using calibration with synthetic isotope mixtures

Dmitry Malinovsky,* Philip J. H. Dunn and Heidi Goenaga-Infante

This paper demonstrates that the combination of simultaneous measurements of $^{12}C^+$ and $^{13}C^+$ ion currents by MC-ICPMS and calibration based on the use of gravimetrically prepared synthetic isotope mixtures provides a unique possibility for accurate determination of absolute $^{13}C/^{12}C$ isotope amount ratios which are metrologically traceable to the SI units. We have applied this approach to the determination of $^{13}C/^{12}C$ isotope amount ratios in glycine – an amino acid of importance in a variety of applications, including paleoecological studies and food security analysis. Eleven synthetic isotope mixtures with different $n(^{13}C)/n(^{12}C)$ isotope amount ratios were prepared by gravimetric weighing and mixing of two well-characterized parent glycines, each highly enriched in either ^{12}C or ^{13}C isotopes. The

Reference Material & Accreditation

- Extension to scope of UKAS accreditation obtained recently
  - ISO Guide 34 Reference materials for glycine RMP
  - ISO/IEC 17025 Testing Laboratory for C isotope ratios by MC-ICP-MS



LGC

ERM European Reference Materials

CERTIFICATE OF ANALYSIS

ERM®- AE672a

Glycine – Absolute carbon isotope ratio		
Constituent	Certified Value[1] (ratio)	Uncertainty [2] (ratio)
$n(^{13}C)/n(^{12}C)$	0.010648	0.000031

1) The certified value is the absolute carbon isotope ratio $n(^{13}C)/n(^{12}C)$ determined at LGC using MC-ICP-MS calibrated with synthetic isotope mixtures, and is traceable to the SI through calibration standards of known purity[2].

2) The quoted uncertainty is the half-width of the expanded uncertainty interval, calculated using a coverage factor (*k*) of 2, which gives a level of confidence of approximately 95 %.

This certificate is valid for 12 months from the date of shipment provided the sample is stored under the recommended conditions.

The minimum amount of sample to be used is 0.2 mg.

NOTE

European Reference Material ERM®-AE672a was produced and certified under the responsibility of LGC according to the principles laid down in the Technical Guidelines of the European Reference Materials® co-operation agreement between BAM-LGC-IRMM. Information on these guidelines is available on the Internet (http://www.erm-crm.org).

Accepted as an ERM®, Teddington, April 2014.

Signed: _____

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Conclusions and Outlook

- The application of carbon isotope ratios for food origin/authenticity determination, discussed here, will benefit from the production of new reference materials.
- LGC's future efforts will focus on the characterisation of matrix-matched reference materials, critical to calibration and validation of C isotope ratio measurements.
- Reference materials certified for **absolute** carbon isotope ratios traceable to the SI have shorter traceability chains than those traceable to VPDB.

Acknowledgements

- Dmitry Malinovsky and Heidi Goenaga-Infante.
- John Warren, Fahmina Fardus and Dipti Dhokia for purity measurements of parent glycines.
- Gill Holcombe and the RM production team.

Acknowledgements

The work described in this presentation was funded by the UK National Measurement System.

National Measurement System

National Measurement Office

NPL National Physical Laboratory

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nel technology for life