HIDEX

Application Note – Liquid Scintillation Counters

Determination of the ^{14}C content in bio-based products – applications, techniques and tools

In brief

- Various techniques can be used for the determination of radioactive carbon (^{14}C) content in bio-based products, including: biofuels, biopolymers, and archaeological objects.
- Accessibility, instrument cost, and various sample preparation methods make liquid scintillation counters suitable for diverse applications.
- Hidex 300 SL and 600 SL (300/600 SL) are next generation LS counters, which uniquely combine modern technology with sophisticated design and user-friendly software.
- The instruments utilize three PMT detectors enabling high counting efficiency and luminescence free counting. In addition, TDCR technology provides automatic quench correction, while excluding the need for external standard methods.

Introduction

In recent decades new bio-based products, such as biofuels, have gained increased popularity. Due to the limited availability of fossil fuels, and shared concern about global warming, there is a growing interest by industry, researchers, and stakeholders in the promise of renewable resources. For example, in the United States and Europe, governments are pushing strongly towards increased use of bio-derived resources (1,2). Besides the environmental advantages, additional benefits can be gained by developing bio-derived products with new functionalities.

Traditionally, radiocarbon determination techniques have been used to estimate the age of archaeological objects. However, these methods are suitable for measurement of bio-based carbon content in other applications as well (see Applications). This application note evaluates how different techniques perform in bio-product analysis.

Applications

- Age determination of archaeological objects by carbon dating
- Analysis of bio-based components in gasoline, bio-ethanol, and bio-diesel
- Determination of bio-based content in biopolymers and bioplastics
- Analysis of environmental samples

Basics of the ^{14}C cycle in nature

In nature, three principal isotopes of carbon occur naturally: stable isotopes ^{12}C and ^{13}C, and the radioactive isotope ^{14}C. Radioactive ^{14}C is formed in the upper atmosphere due to cosmic radiation: nitrogen (^{14}N) captures a high-energy neutron and undergoes a further conversion to form an unstable ^{14}C atom and a proton:

Then, ^{14}C is oxidized to $^{14}CO2$, which is then absorbed by plants during photosynthesis. Importantly, the intake of ^{14}C terminates immediately when the organism dies, and the constant decay of ^{14}C back to ^{14}N and a beta particle begins:

Thus, the ratio between ^{14}C and $^{12}C/^{13}C$ in a deceased organism decreases at a constant rate over time. Due to its half-life of 5,730 years, ^{14}C is almost absent from fossil products older than 20,000 to 30,000 years (4).

Standard methods for ^{14}C determination

ASTM D6866-12 (updated in 2012)

- Accelerator mass spectrometry
- LSC combined with benzene synthesis

CEN/TS 16640:2014 (updated in 2014)

- LSC with sample pre-preparation methods or direct measurement
- Beta-ionization
- Accelerator mass spectrometry

DIN-51637 (updated in 2014)

- LSC for determination of ^{14}C in diesel fuels and middle distillates

Analysis of ^{14}C content

Bio-based carbon content can be determined by various radiocarbon methods:

- Liquid scintillation counting (LSC) is an indirect method, which is used to observe beta particles derived from ^{14}C decay. During LSC the emitted beta particles interact with an organic solution containing scintillation molecules. This results in photon emissions, which can be measured. Due to the presence of low concentrations of ^{14}C in the atmosphere, the LS counter must meet certain technical aspects to compensate.
- Beta-ionization (BI) is also an indirect method for beta particle detection. Unlike LSC, BI counting occurs in a gas counter, where beta particles are measured as electrical pulses between high-voltage electrodes. However, this method is not widely used for analysis of bio-based products due to a counting time of several days, and limited access to BI instruments.
- Accelerator mass spectrometry (AMS) measures the presence of ^{14}C isotopes directly. After the sample is reduced to graphite, its atoms are converted into high-energy ions, and accelerated on an electric field to capture accurate ^{14}C counts. Although AMS is a highly sensitive method, the complexity of the technology, as well as its cost, limit the usability.

Preparation for ^{14}C measurement in LSC

Before analysis, the carbon must be converted into carbon dioxide (CO_2) by combustion in oxygen. Depending on the sample type and instrument specifications, the CO_2 is then collected and prepared for measurement using methods including:

- Absorption in carbamate solution
- Absorption in NaOH solution followed by transfer to carbamate solution
- Direct conversion to benzene

In addition, LSC is suitable for direct measurement of samples such as bio-ethanol and bio-oils, if dissolved in organic scintillation solution. When applicable, direct measurement saves both time and resources by offering a cost-effective option for bio-based carbon analysis.

Reporting the ^{14}C content

Depending on the application, the amount of bio-based carbon can be expressed as a fraction of sample mass, total carbon content, or total organic carbon content (4). For the calculation, a reference value of 105 pMC (percentage of modern carbon) to 100% bio-based carbon should be used. This takes into account the current ratio between atmospheric $^{14}C/^{12}C$, which has been affected by atom bomb testing in the 1950s and fossil fuel consumption during the last century (3).

Comparison of methods

According to the ASTM standard, the maximum total error for both AMS and LSC is ±3% (3). Also, direct comparisons between the methods with differently processed biofuel samples have yielded comparable results (Figure 1) (5). Thus, other issues such as the cost, accessibility, and speed of the analysis should be taken into account when selecting the optimal radiocarbon method for the bio-content analysis (Table 1, 2).

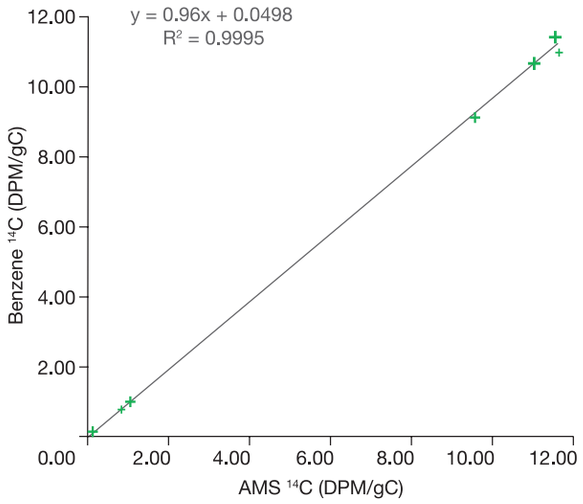

Figure 1. Comparison of the ^{14}C activity in biofuels analyzed by LSC coupled with benzene synthesis, or by AMS. Activity of ^{14}C in various biofuel samples indicates good agreement between the measurements done by AMS and LSC. Activity is indicated as disintegrations per minute per gram carbon (DPM/gC). Data is derived from Culp et al. 2014.

Table 1. Technical comparison between LSC and AMS for ^{14}C content detection in bio-based products (4,6).

Method	Liquid scintillation counting (low level counter)	Accelerator mass spectrometry
Technical level	Simple: suitable for normal laboratories	Very complex: needs specific expertise and laboratory
Additional requests	Small to medium space request; optional device needs depending on the sample preparation method	Large space request; need for a graphite conversion device
Costs	Low (instrument) Low (analysis)	High (instrument) Moderate (analysis)
Sample preparation time	0 to 6 h, depending on the method	2 h
Measurement time	1 to 12 h	10 to 30 min
Contamination potential during the process	Low to moderate	High

Table 2. Advantages and disadvantages of LSC and AMS for ^{14}C content detection (4–6).

Liquid scintillation counting	Accelerator mass spectrometry
Advantages ▪ Good instrument availability, suitable for normal laboratories ▪ Lower cost both for the instrument and per analysis ▪ Various sample preparation methods available ▪ Immediate analysis with direct measurement can be used for liquid samples ▪ Higher amount of the sample enables better representativeness ▪ Lower risk for contamination of the sample	**Advantages** ▪ Very high sensitivity ▪ Precise analysis ▪ Low amount of sample needed, which can also be a drawback
Disadvantages ▪ Need for a low level counter to overcome background issues ▪ For some sample preparation protocols, additional devices needed	**Disadvantages** ▪ Complex technology, need for specific expertise and laboratory ▪ Need for a graphite conversion device ▪ High cost for the instrument ▪ Higher risk for contamination of the sample ▪ Lower amount of the sample is associated with poor representativeness, and higher number of replicates needed

HIDEX 300/600 SL - AUTOMATIC TDCR LIQUID SCINTILLATION COUNTERS

Hidex LS counters with three photomultiplier tube (PMT) detectors are suitable for bio-content determination in various sample materials. Modern design, small size, and easy installation make Hidex 300 SL suitable for any laboratory. For high throughput needs, the Hidex 600 SL offers an effective option with technology similar to the 300 SL series. The low level models with active guard meet international standards criteria for LS counters.

High counting efficiency

In comparison to traditional LS counters, with one or two PMT detectors, Hidex 300/600 SL provide optimal counting geometry with three PMTs. This enables higher counting efficiency, which is especially useful for low energy isotopes and highly quenched samples.

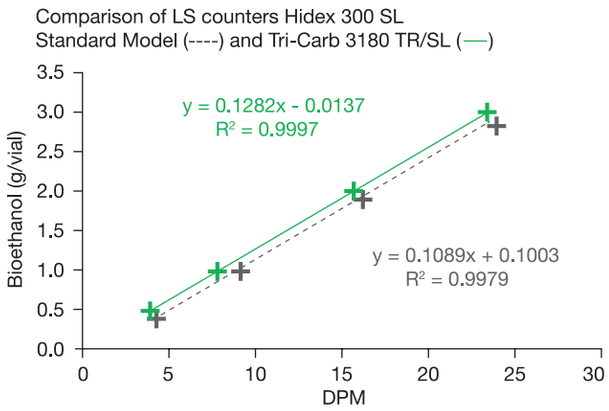

Figure 2. Analysis of the ^{14}C content from bioethanol with direct LSC measurement. Different quantities of ethanol (0.52–3.0g) were mixed with water (3.0g) and Hisafe 3 cocktail to a total volume of 15ml. Samples were analyzed with Hidex 300 SL standard model or Tri-Carb 3180 TR/SL using direct counting for 90min (counting window 5–650; coincidence time 70ns). The instruments gave comparable results, and by selecting the low-level model of Hidex 300 SL, one could obtain even lower background counts.

Performance parameters set for the LSC

	Criteria ASTM D6866-12	Hidex 300/600 SL
Bkg	< 5 DPM	< 4 DPM
Efficiency	> 60%	> 85%
FOM (Eff²/Bkg)	> 1000	> 2000

Automatic quench correction by TDCR

Three PMTs enable integration of triple-to-double coincidence ratio (TDCR) counting, as well as automatic quench correction which is suitable both for chemical and color quenching. Technically, TDCR takes into account both the double and triple counts from the PMTs, and calculates the ratio between them:

$$TDCR = \frac{C_t}{C_d + C_t} = \frac{C_t}{C_{all}}$$

TDCR: Triple-to-Double Coincidence Ratio
c_t : triple coincidence counts
c_d : double coincidence counts
c_{all}: all coincidence counts

TDCR is relative to counting efficiency, resulting in disintegrations per minute (DPM) with a good accuracy of ±10%. Furthermore, curve-fitting functions can be utilized together with TDCR to improve its accuracy up to ±2%. More generally, the Hidex 300/600 SL counters provide separate acquisition of triple and double coincidence spectra, thus allowing the most comprehensive quench information.

Luminescence free counting mode

Because triple counts from PMTs are not affected by chemiluminescence, even highly luminescent samples, including bio-oils, can be analyzed immediately using the triple counting mode of Hidex 300/600 SL counters (Figure 2, 3). This saves time since no dark adaptation of the sample is needed. In addition, the analysis is more reliable when luminescence-derived interference is excluded.

Measurement procedure

In direct measurement, samples will have a variable degree of chemical and color quenching. Thus, a reliable quench correction method is critical for successful analysis. On Hidex 300/600 SL counters, quench correction can be done from luminescence-free counts using TDCR algorithms without the need for an external standard source. Alternatively, quench correction can be done by using an external standard source, if provided.

In the measurement of pre-processed samples (e.g. benzene synthesis), quenching is constant from sample to sample because all samples are processed with the same method. The preferred quench correction option with Hidex 300/600 SL is the utilization of the constant quench correction factor, and the use of luminescence-free count mode.

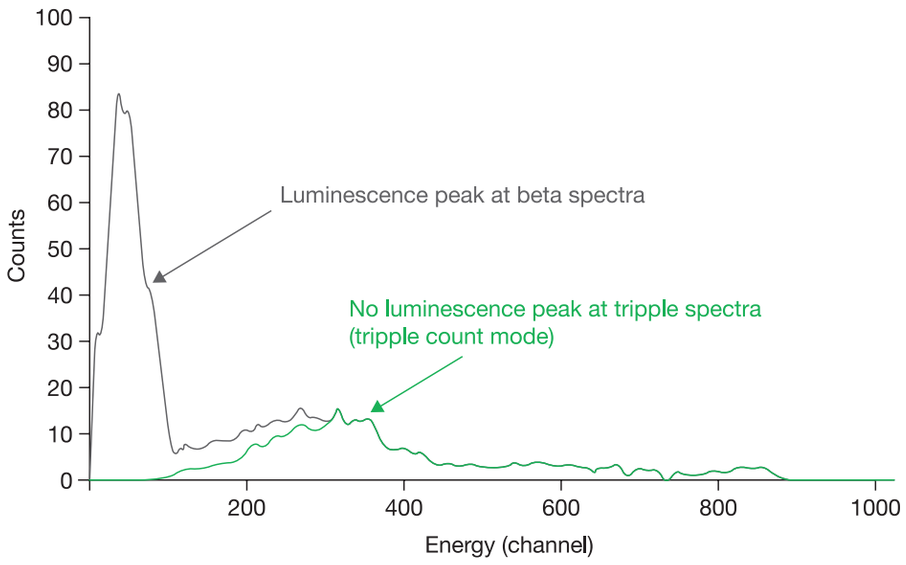

Figure 3. Direct detection of bio-oil fraction with triple count mode. Bio-oil samples with variable degrees of color luminescence interference were analyzed using direct measurement on Hidex 300 SL. 10ml oil-samples were mixed with 10ml MaxiLight cocktail, and the samples were counted for 100min (counting window 110–580). (A) A luminescence interference peak appeared in double counts (black spectra), but did not interfere when triple counting mode was utilized (green spectra).

Sophisticated software

The Hidex 300 SL and 600 SL are operated using an external PC, which is simple to update or replace without needing to purchase a new instrument. In addition, the sophisticated MikroWin 300 SL software provides an easy to use interface and data export functions. Extensive data reduction features, including quench curve analysis and half-life correction, make this software adaptable to users' specific needs.

Hidex 300/600 SL benefits

- Three PMT detectors enable high counting efficiency with minimum interference from luminescence or quenching
- Automatic quench correction by TDCR excludes the need for an external standard source, and corrects both chemical and color quenching
- Luminescence-free counting facilitates immediate measurement without the need for dark adaptation of samples
- Compact design and cost-effectiveness make the instrument suitable for any laboratory
- User-friendly software adapts to tailored analysis and data export needs

References

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6. Noakes J, et al. A Comparison Of Analytical Methods For The Certification Of Biobased Products. Radiocarbon. 2007;49:259–72.

Hidex Oy is a family owned high-technology company which develops and manufactures high-performance analysis equipment for the specific needs of industry, healthcare providers, and academic researchers. Hidex know-how stems from decades of experience with liquid scintillation counters, and continuously developing LSC technology in close collaboration with customers and the scientific community. In this way, users' access to the most innovative products and software is ensured into the future. Hidex also offers extensive support, so that customer can focus on analyses.

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