LSC
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Determination of the ^{14}C content in fuels containing bioethanol and other biogenic materials with liquid scintillation counting

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Introduction

The limited resources of fossil energies such as coal, oil and gas are generally known. These resources will only be able to deliver the necessary amount of energy within the next few decades. Especially the heavily increasing use of fossil sources in Asia and other rapidly growing markets and the already high level of burning fossil sources in the western industrial nations will result in a shortage of these essential materials. We already made the experience that the increasing use of fossil sources followed a strong increase in prices for consumers. These lead to a search for alternative energy sources during recent years.

Another challenge is the attempt to reduce the emission of CO_2 to avoid a further increase of the temperature in the atmosphere. Carbon dioxide has been generally accepted as one potential source of the green house effect although we still need further information to fully understand the complex mechanisms that result in the global temperature increase. To stop this increase in temperature many countries agreed in the Kyoto protocol in a CO_2 reduction of their emissions over the next years.

One possibility to make additional source of energy available for a longer time and to reduce the emission of fossil carbon dioxide is the use of renewable (biogenic) sources.[1)] The production of energy from sugar cane, rape, corn and other biogenic materials is far away from the research phase and a number of biogenic products will already be added to fossil fuels.

The regulation 2003/30/EC from the EU determines the minimum amount of biogenic materials in fuel. Until 2005 all fuels should contain at least 2% of biofuels and this should increase until 2010 to 5.75%. The current European norm for Otto fuels is EN DIN 228 which already allows the use of up to 5% of bioethanol. For diesel the corresponding norm is EN DIN 590, for biodiesel the current norm is EN DIN 14214, which has been introduced in Germany on the 30th of October 2004.

This application note will show in more detail the possibilities to determine the amount of biogenic materials in mixtures of fossil and biogenic materials with the help of the liquid scintillation counting (LSC) method. A very accurate method for the quantification of the biogenic amount in fuels is very important for producers as well as for custom departments in the different countries.

What is the basic principle of the quantification of biogenic material?

Living organisms take up carbon with their food or via breathing or photo synthesis. During these processes different carbon isotopes such as the stable nuclides ^{12}C and ^{13}C as well as the radioactive nuclide ^{14}C will be incorporated in organic material in the exact same proportions in which they occur in nature.

We can assume that the amount of the radioactive nuclide ^{14}C in the atmosphere is constant during the growth period of plants because the production of ^{14}C via neutron capture of ^{14}N is in equilibrium with the radioactive decay of ^{14}C. This is true as long as the plant growth is fast compared to the ^{14}C activity fluctuations in the atmosphere. Most plants for biofuel production will be harvested within one year and are therefore not influence by long term ^{14}C activity changes. Trees which might grow over decades can show higher amounts of ^{14}C in the tree rings of the 60th due to the atom bomb testing. As long as a living organism takes up carbon we have an equilibrium activity of ^{14}C because decay and uptake of ^{14}C is in equilibrium.

As soon as an organism dies or you harvest a plant the uptake of carbon stops. From this point on the original amount of ^{14}C decays and the current activity of this material is only dependent on the half life of this isotope. Because ^{14}C has a half life of 5730 years half of the original activity will be decayed after 5730 years. Currently the most sensitive detection methods for ^{14}C can detect 14C even in samples which are already 10 half life's old, which is approximately an age of 60 000 years. In older samples ^{14}C can not be detected anymore.

Because in fossil materials or in products prepared from fossil materials such as all mineral oil products the ^{14}C contents could decay over million of years no ^{14}C can be detected anymore. On the other hand in biogenic material all ^{14}C is still present. This difference in 14C activity can be used to determine the amount of biogenic material in fuel. Figure 1 demonstrates the ^{14}C circle in nature.

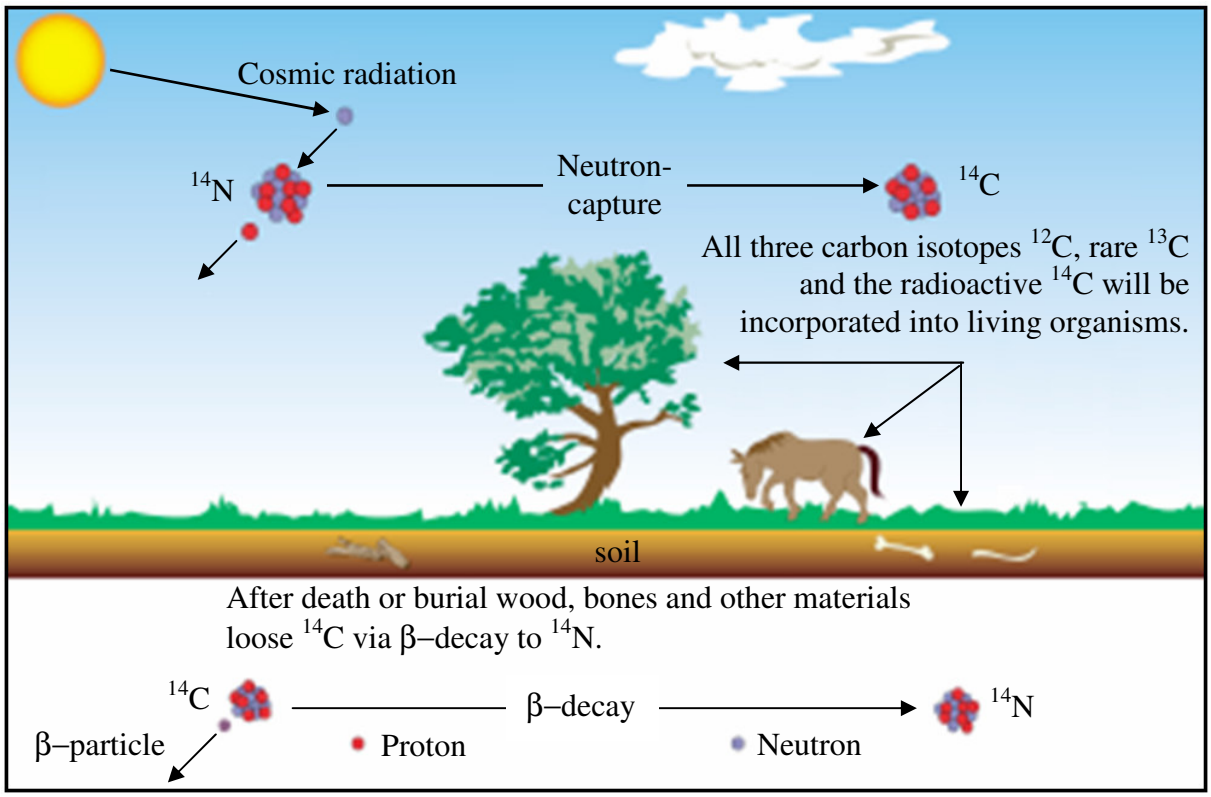


Figure 1: Production and incorporation of ^{14}C in organic matter

An assumption we have always to make is that samples must only contain mixtures of fossil and biogenic materials. A contamination with older ^{14}C samples (for example from trees) should be avoided.

To allow inter laboratory comparisons and comparisons between samples from different time periods result will be published in many cases as % m or % mc (% modern or % modern carbon).

In this case the amount of ^{14}C atoms will be determined relative to the year 1950.[2)]
As a reference material a sample from 1950 will be used which showed an activity of 13.56 ± 0.70 DPM/g carbon.[3,4)]
If you do not determine the amount of ^{14}C in your samples relative to 1950 but as a percentage of the current ^{14}C activity you have to know that this includes a higher activity due to the atom bomb tests. In this case you have to use an activity of 14.62 DPM/g carbon.[5)]

Methods to determine the amount of biogenic material

In general two methods can be used which are sensitive enough to detect low activities of ^{14}C. Both methods will be described in detail in ASTM method D 6866-06 and can be downloaded from www.astm.org.
One method describes the use of AMS (Accelerator Mass Spectrometry) or IRMS (Isotope Ratio Mass Spectrometry).
The other method uses liquid scintillation counting. We will concentrate on the following pages on this latter method. The LSC technology allows using three different procedures for the determination of 14C in fuel:

Method A: Measurement of CO_2 in a LSC.
Method B: Use of a mass spectrometer.
Method C: Measurement of benzene in a LSC.
Method D: Direct measurement of the organic sample in a LSC.
Method A and C are especially interesting for liquid scintillation counting in case the sample has been prepared by sample combustion or sample burning. In case of method C the resulting carbon dioxide will be converted via several steps into benzene. As a consequence of this reaction you can get much higher carbon content in your sample resulting in much higher sensitivity. Also benzene is already a very good solvent for LSC measurements and you only add scintillators to your sample allowing you to make full use of your vial volume for your sample. However, it should be mentioned here, that the use of the benzene synthesis method needs a high degree of experience with this method and usually it is not possible to introduce this method in a laboratory right away.
The direct measurement of an organic sample in the LSC is always advantageous if a sample such as biofuels can be dissolved in the scintillation cocktail in any possible ratio. The organic sample should also show no or only little colour and the amount of biogenic material should be in the range of at least 1% (in case 50% carbon in the sample). Carbon content of below 1% would result in extremely long counting times or large standard deviations. Advantages and disadvantages of the four different methods will be explained in Table 1.

Method	Advantage	Disadvantage
Method A: CO_2 in LSC	Less sample preparation and low costs compared to method C, good instrument availability.	Low sample activity due to limited sample capacity of CarboSorb E. Not sensitive for lowest ^{14}C activities.
Method B: AMS	High sensitivity, very precise.	High costs, therefore mainly for samples with carbon content below 10%.
Method C: Benzene in LSC	High sensitivity, very precise, good instrument availability.	More time consuming sample preparation, low capacity, benzene is cancerogenic material.
Method D: Direct measurement in LSC	Minimum, very fast sample preparation, good sensitivity, low costs per measurement, good instrument availability.	Keine offiziell standardisierte Methode nach ASTM 6866-06 verfügbar.

Table 1: Comparison of Advantages and Disadvantages between methods A – D.

So far no standardized methods are available for method D (no part of method ASTM D 6866-06) although the use of this method for biofuels is obvious. In the meantime some investigations clearly show that method D is a suitable method for the quantification of biogenic material.[6, 7, 8)]

For further information about method A and C please read the literature.[9, 10)] Table 2 illustrates so approximated costs and necessary time for the different methods.

Method	Sample preparation	Time (Min.)	Analysis costs	Instrument costs	Sample size	Risk of contamination	Precision
A	3 Stunden	1300	250 $	150 K$	0,2-1 g	medium	< 9%
B	2 Stunden	20	400 $	2 M$	1 mg	high	< 1%
C	3 Stunden	1300	250 $	150 K$	2-10 g	low	< 2%
D	3 Minuten	330	150 $	100 K$	5-15 g	low	< 3%

Table 2: Differences between methods A – D.

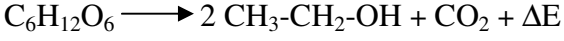
Other technologies, not using liquid scintillation technology, such as chromatographic or IR-spectrometric technologies can be used to identify and quantify ethanol or FAME but they can not distinguish between biogenic ethanol or FAME and synthetic, fossil ethanol or FAME. This can only be done with the help of scintillation technology or mass spectrometry. On the following pages we will discuss method D for the quantification of Biofuels.

What kind of bio materials will be measured?

In fuels ethanol, ETBE (Etyhl-tert-butylether) and MTBE (Methyl-tert-butylether) are the most common bio additives. In diesel fuel FAME (fetty acid methylester), RME (rape methylester), BTL (Biomass to liquid) and GTL (Gas to liquid) are the most often used bio additives.

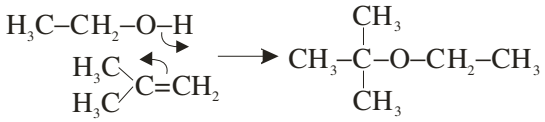
How do we produce biofuels?

In normal Otto fuel mainly bioethanol and ETBE is used. Bioethanol originates from the alcoholic fermentation of sugars:



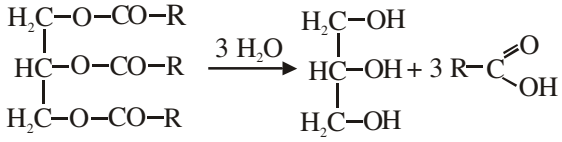
Sugars are mainly made out of sugar cane, sugar beet or especially in Germany from corn. Sugars are produced by enzymatic or acid induced cleavage from starch molecules.

ETBE will be produced from Isobuten and bioethanol via an addition reaction:

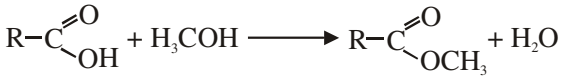


(EtOH + Isobuten) (ETBE)

Biodiesel mainly consists of FAME (Fatty acid methylester) produced from rape why it is also called RME (Rape methylester). The fatty acid which are available from rape can not directly be used for traditional engines in most cases, because their viscosity is very high. RME almost exclusively consists of fatty acid esters of glycerol. These tri-glycerides have to be cleaved in oil refineries where the cleaved fatty acid will be converted into the corresponding methylester.



Tri-glycerid ⟶ glycerol + fatty acid



Fatty acid + methanol ⟶ FAME

This chemical procedure is necessary because tri-glycerides show properties which are unwanted in engines.

Some tri-glycerides are very viscous or even solids (for example bovine tallow) and can especially not used at low temperatures in classical engines. Esters with multiple double bonds such as linol or linolenacid can be oxidized by air and tend to show radical polymerization.

Principle of scintillation counting:[9)]

Liquid scintillation counter measure the radioactivity via indirect measurement of light with the help of photo multipliers (PMT´s). The light is a results of an interaction between ionizing radiation and a so called cocktail[11)] which will be added to the sample. As you will see soon we need extremely sensitive instruments for the detection of natural radioactivity. The TriCarb 3170TR/SL uses in addition to coincidence technology and patented time resolved measurement technology[12, 13)] a guard-detector made out of bismuthgermanate (BGO). The combination of these technologies results in extremely high sensitivity due to a drastically reduction of background pulses without sacrificing counting efficiency. Figure 2 illustrates the TriCarb surround-guard-detector.

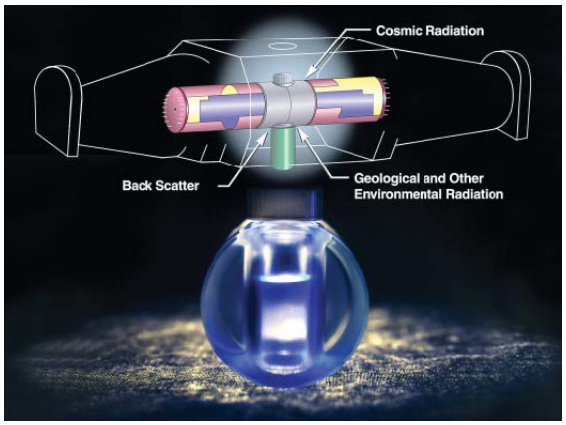


Figure 2: BGO-detector in TriCarb 3170

Another very sensitive instrument which can be used for this application is the Quantulus from PerkinElmer. This instrument uses very efficient lead shielding (630 Kg) in combination with an anti-coincidence circuit which also allows extremely low background values.

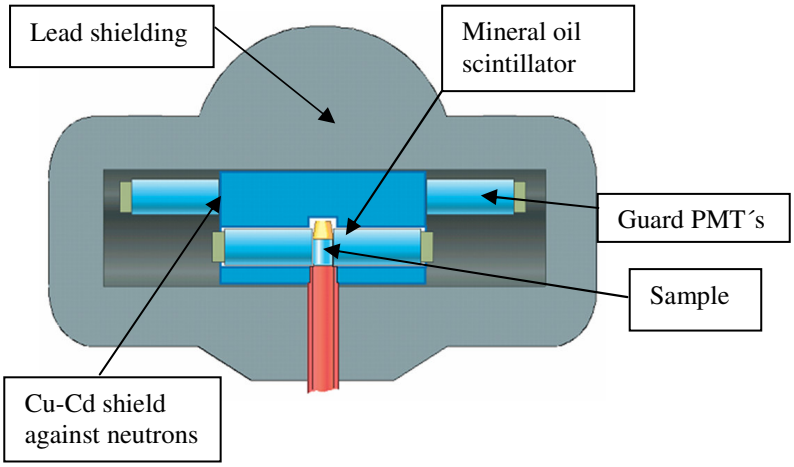


Figure 3: Quantulus shielding and Guard-PMT´s

Both systems offer high sensitivity and offer latest quench correction methods. The instrument that best suits your individual application should be determined during a discussion with you and one of PerkinElmer's specialists.

What is the necessary sensitivity of the scintillation counter?

To answer the question about the minimum sensitivity of a liquid scintillation counter for the measurement of biogenic samples we have to estimate the expected activity in such a sample. Because biodiesel and bioethanol are available in large quantities we should use a much sample as possible for the measurement in the LSC to increase the activity. With an optimized cocktail we should be able to use cocktail sample ratios of 1:1 (cocktail:sample) or even a slight excess of sample (possible with bioethanol) because the sample is of purely organic nature. Undiluted biodiesel can show a significant yellow colour but because currently maximum content in biodiesel for traditional engines is in the range of 5% the colour will be heavily diluted thus reducing colour quench significantly allowing direct measurement of these samples.

The purely organic nature of biodiesel allows the use of cocktails without emulsifying additives resulting in a better performance of the cocktail. A typical diesel fuel currently contains approximately 5% biodiesel. A measurement vial with 10g of diesel fuel contains approximately 0.5g biodiesel.

The carbon content in such a mixture is roughly 86% resulting in a carbon amount of biogenic material of 0.43g. This carbon contains mainly the non radioactive carbon isotope ^{12}C and only a very small amount of the radioactive isotope ^{14}C. Among one billion ^{12}C nuclides we find less than one ^{14}C nuclide. In the sample of less than 1 gram of carbon (0.43g) which we want to investigate we have the unimaginable amount of less than one billionth of a gram ^{14}C. Nevertheless we can detect even such small amounts of activity. In one gram carbon we have 14.62 decays in every minute (related to the current specific activity of natural carbon). This means that we have 6.3 decays per minute (6.3 DPM or 0.1 Bq) in our sample containing 0.43g carbon. We now know the approximate activity of our sample. What kind of scintillation counter do we need fort hese activities. We can use the DIN formulas to calculate the detection limit and the critical level of detection.[14)] To calculate the deteciton limit we need several values. The measurement time has a significant influence on the detection limit; we have to know the background of our system and the sample volume as well as the counting efficiency. From all these values we can determine the detection limit. In this application note we use formulas from DIN norm 25482. For details about counting statistics and error calculation please also read application note 25[14)].

Target: Measurement of a sample containing 6.3 DPM.

To check if our scintillation counter is suitable fort his method we first have to determine the critical detection limit:

$$g^* = \frac{k_{1-\alpha}}{E \cdot V}\sqrt{R_0 \cdot \left(\frac{1}{t_0}+\frac{1}{t_m}\right)}\ \text{Bq/L}$$

The sensitivity of the scintillation counter can be determined using the formula for the detection limit:

$$g = \frac{k_{1-\alpha}+k_{1-\beta}}{E \cdot V}\sqrt{R_0 \cdot \left(\frac{1}{t_0}+\frac{1}{t_m}\right)}\ \text{Bq/L}$$

The values $k_{1-\alpha}$ and $k_{1-\beta}$ include errors of 1. and 2. order. The values t_0 and t_m are measurement times for background and sample. In case both counting times are identical the formulas can be simplified as follows for the critical level of detection:

$$\boldsymbol{g^* = \frac{k_{1-\alpha}}{E \cdot V}\sqrt{\left(\frac{2R_0}{t}\right)}}\ \text{Bq/L}$$

and for the detection limit:

$$\boldsymbol{g = \frac{k_{1-\alpha}+k_{1-\beta}}{E \cdot V}\sqrt{\left(\frac{2R_0}{t}\right)}}\ \text{Bq/L}$$

Biofuels can be measured in LSC´s without strong quench. Only samples containing FAME have to be diluted with excess of cocktail to reduce colour quench. Because no water is present in the samples we can use a pure organic cocktail such as Ultima Gold F resulting in very high counting efficiencies and high uptake capacities. A sample of 11ml diesel and 8 Ultima Gold F with a counting efficiency of 75% in a TriCarb 3170TR/SL with 1.5 CPM Background (0,025 CPS) and a measurement time of one hour (3600 Sekunden) and a $k_{1-\alpha}$ value of 3.0 and $k_{1-\beta}$ value of 1.645 will result in a critical level of detection of:

$$\mathbf{g^* = \frac{3}{0.75 \cdot 0.011}\sqrt{0.025 \cdot \left(\frac{1}{3600}+\frac{1}{3600}\right)}}$$

$$\mathbf{g^* = 1.36}\ \text{Bq/L}$$

The critical level of detection is 1.36 Bq/L which is equal to 0.08 DPM/ml or 0.9 DPM/vial. As we want to determine an activity of 6.3 DPM in our vial which is much more than the critical level, the LSC can be used for this method.

For the detection limit we can calculate:

$$\mathbf{g = \frac{4.645}{0.75 \cdot 0.011}\sqrt{\left(\frac{2 \cdot 0.025}{3600}\right)}}$$

g = 2.1 Bq/L

The detection limit is 2.1 Bq/L which is equal to 0.13 DPM/ml or 1.38 DPM/vial. Only higher activities can be detected which is the case with our sample.

Because some countries have lower tax for biofuels there is large interest in the accurate quantitative determination of biofuels. The statistical precision of the obtained results is therefore of major importance for this application.

Experimental part:
The following measurements have been done with a TriCarb 3170TR/SL or the Quantulus, both from PerkinElmer. The evaluation of spectral data has been done with the SpectraWorks evaluation software. The cocktail used was Ultima Gold F (PerkinElmer Art. Nr. 6013179) and the vials were High Performance Glas Vials, 20ml (PerkinElmer part no. 6000128 or 6000134) or Teflon coated plastic vials (6000477). If not mentioned otherwise 10ml cocktail and 10ml sample have been used for the measurement. At this amount of cocktail colour quench was significantly decreased in biodiesel samples. Samples with bioethanol only did not show any colour quench. Recent experiments showed that better results can be obtained with sample cocktail ratios of 12:8.
Figure 4 illustrates four LSC spectra. The measurement time was always 1200 minutes. Spectrum **(a)** is a background measurement, spectrum **(b)** is pure bioethanol, spectrum **(c)** is Ultimate diesel fuel without any biodiesel, and **(d)** is 100% FAME in Cocktail. In spectrum **(b)** we can easily see the excellent ^{14}C signal up to an energy of approximately 60 keV. The low energy shift is typical and due to the chemical quench of the alcohol.

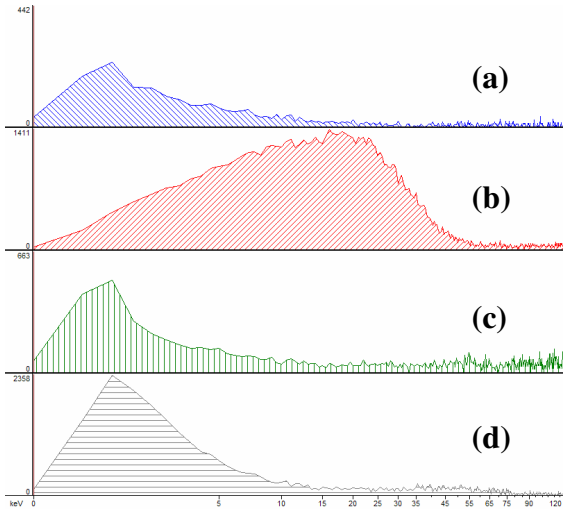


Figure 4: Different fuels[7)]

In spectrum (c) which is biodiesel free Ultimate fuel from Aral we do not see much activity as we expected. The activity is only slightly above background. This fuel is only based on fossil fuels and originally present activity should be decayed until today. As we could prove the signal between 0 and 4 keV is due to chemiluminescence. Keeping the samples overnight in the dark before starting counting could eliminate the luminescence.

^{14}C Counts in energy window 4-120 KeV		
Fuel	**Counts**	**tSIE**
Background	939	-
Bioethanol	46779	307
Ultimate diesel	1896	644
FAME	4798	15

Table 3: Measurement results[7)]

Heavy chemiluminescence could be detected with FAME samples. Cooling helped to reduce the level of luminescence in these samples. In ^{14}C samples chemiluminescence can be eliminated in most cases by reducing the energy window. Luminescence is a very low energy signal. Starting the measurement at 4 keV instead of 0 keV eliminates luminescence almost quantitatively without reducing the counting efficiency to much.
The lower „Counts“ value of FAME compared with bioethanol is mainly a result of the strong colour quench.

With a tSIE of 15 in this case a quantitative determinationn of the absolute activity is only possible with colour quench correction. In realistic fuel samples the amount of FAME usually does not exceed 10% resulting in a much lower colour quench. The following figure clearly shows that colour quench in FAME samples will be drastically reduced due to the increasing amount of cocktail.

Figure 5 illustrates the same samples (FAME) but in different ratios with cocktail. The bottom spectrum with only 0.5 ml FAME and 19.5 ml cocktail shows a significant shift of the spectrum to higher energies because quench has been eliminated due to the dilution with cocktail.

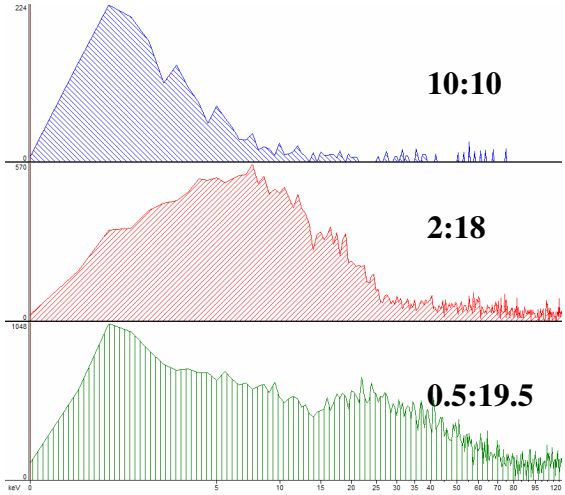


Figure 5: FAME depending on dilution with Cocktail[7)]

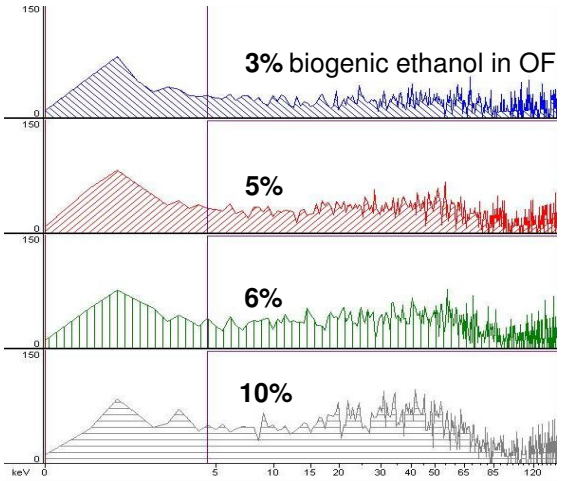


Figure 6: ^{14}C spektra in Otto-fuel (OF) with different amounts of bioethanol.[7)]

Figure 6 illustrates spectra of Otto-fuel with different amount of bioethanol. As shown in table 4 the quench parameter is almost constant in biofuel and with a value close to 500 the quench is only weak in contrast to the data for FAME in table 3. Colour quench is practically absent in bioethanol samples and therefore even CPM data allow a good determination of the amount of bioethanol in fuel.

^{14}C Counts in energy window 4-115 keV		
% Bioethanol	**Total counts**	**tSIE**
3	1420	527
5	1862	509
6	2024	501
10	2737	499

Table 4: Results from OF with different bioethanol content[7)]

The quench in bioethanol samples is exclusively due to chemical quench in the sample. This quench was more or less constant allowing the use of CPM measurements for a good correlation of measured counts and the amount of bioethanol in the fuel as illustrated in figure 7. We also measured bioethanol samples at the Finnanzlandesdirektion Vienna using the TriCarb 3170TR/SL. Here we used samples with even higher amount of bioethanol to investigate the influence of quench ot higher concentrations of alcohol. These measurements were performed with Teflon coated plastic vials. Usually plastic vials show better transmission for photons, lower reflection and lower background values due to the small amount of ^{40}K.

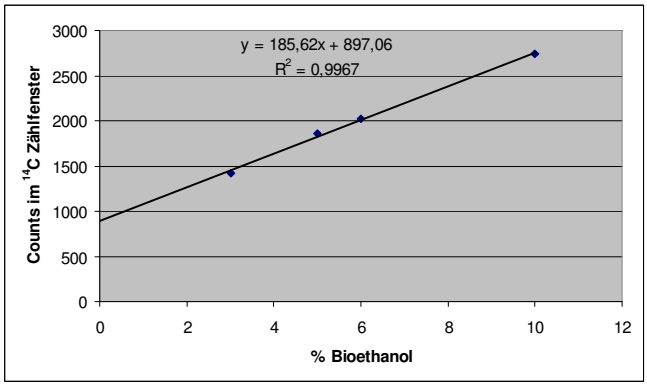


Figure 7: Linearity of CPM measurements[7)]

The optimum energy window was determined using the SpectraWorks software. For bioethanol in fuel we used an energy window ranging from 5-50 keV. Significant luminescence could be detected immediately after mixing ethanol and fuel. For this reason we left samples overnight in the dark inside the instrument before starting the measurement.

CPM	CPM-Background	% Bioethanol	Time in minutes	Total counts	DPM	tSIE	Efficiency %
1,1	0,0	0	80	87	2,3	474,65	–
4,8	3,7	10	480	2303	5,4	419,60	68,5
19,5	18,4	50	480	9355	26,6	360,36	69,2
36,9	35,8	100	480	17707	52,7	318,91	67,9

Table 5: Measurement of bioethanol in the energy window from 5-50 keV[15)]

As you can see the tSIE-value is decreasing significantly reflecting the increasing quench at higher concentrations of bioethanol. This however has almost no influence on the counting efficiency which only varies between 69.2 and 67.9 %. Therefore even CPM-measurements can be used to determine the amount of bioethanol in fuel. Figures 8 and 9 clearly indicate that in addition to DPM determinations simple CPM measurements can be used to quantify bioethanol in fuel. Thus the measurement of external standards can be avoided as long as no other quencher or colour is present.
This is of course impossible in the case of FAME because this sample shows strong yellow colour which may result in a strong decrease in counting efficiency. The use of bromine seems to reduce some colour of FAME samples as indicated by a first experiment.

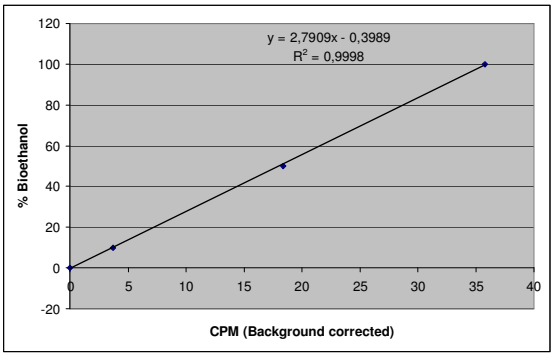


Figure 8: Linearity of the CPM measurement[15)]

Bleaching with oxidizing chemicals (which has to be done very carefully using explosion protection) or the use of active carbon does not result in significant colour reduction.

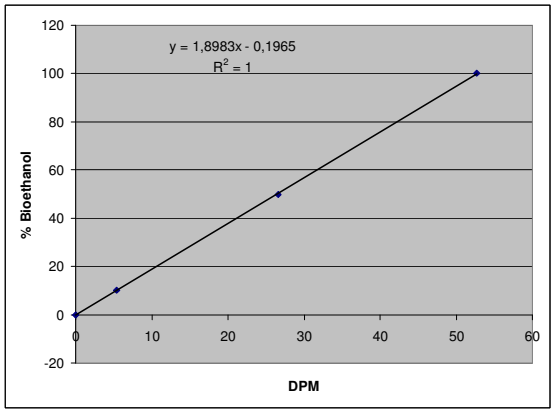


Figure 9: Linearity of the DPM measurement[15)]

Besides the use of the TriCarb 3170TR/SL we also used the Quantulus in our low level laboratory in Turku which is also a very sensitive instrument for the measurement of small biofuel components. As a result of this experiments a first paper has been published recently.[6)] Figure 10 illustrates the good correlation between the concentration of biomaterial and the obtained results. In this publication the authors also mention that biofuels can be mixtures of samples such as bioethanol and ETBE. In such a case calibration curves have to prepared for each component and it is necessary to know the exact composition of the fuel to do accurate quantifications.

The determination of the exact fuel composition can be done using methods such as GC-MS or NMR. It is also possible that components will be prepared from fossil and biogenic materials. For example ETBE can be prepared from bioethanol by addition reaction to fossil isobutene.

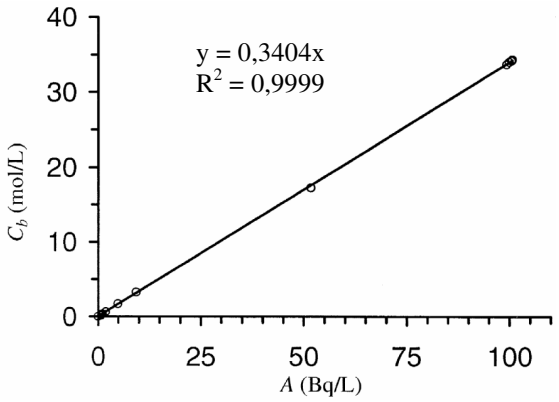


Figure 10: Linearity of DPM measurements in the Quantulus[6)]

Figure 10 illustrates the concentration of bioethanol in mol/L versus the activity. The measurement time in this case was 5.5 hours.

Discussion of the results:

The first measurements clearly indicate that the LSC technology especially using instruments such as the TriCarb 3170TR/SL and the Quantulus are superb instruments for the investigation of biogenic components in fuel. The quantification of biofuel is possible. Due to the low activities in these samples and measurement times in the order of 5 to 8 hours per sample special super low level scintillation counters are required.

For the future it would be helpful to find better ways to reduce the colour of biodiesel samples and still be able to do direct measurements of biofuel. Alternative LSC methods which are also available are sample combustion and the measurement of CO_2 converted into carbamate or the conversion of CO_2 to benzene as discussed above in Table 1.

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