Determination of Hydroxyl Groups in Pyrolysis Bio-oils using $^{31}$P NMR

Laboratory Analytical Procedure (LAP)

Issue Date: March 2, 2016

Mariefel V. Olarte, Sarah D. Burton, Marie Swita, and Asanga B. Padmaperuma
Pacific Northwest National Laboratory

Jack Ferrell and Haoxi Ben
National Renewable Energy Laboratory
Determination of Hydroxyl Groups in Pyrolysis Bio-oils using $^{31}\text{P}$ NMR

Laboratory Analytical Procedure (LAP)

Issue Date: March 2, 2016

Mariefel V. Olarte, Sarah D. Burton, Marie Swita, and Asanga B. Padmaperuma
Pacific Northwest National Laboratory

Jack Ferrell and Haoxi Ben
National Renewable Energy Laboratory

Prepared under Task Number BB143810
NOTICE

This report was prepared as an account of work sponsored by an agency of the United States government. Neither the United States government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or any agency thereof.

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications.

Available electronically at SciTech Connect http://www.osti.gov/scitech

Available for a processing fee to U.S. Department of Energy and its contractors, in paper, from:

U.S. Department of Energy
Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831-0062
OSTI http://www.osti.gov
Phone: 865.576.8401
Fax: 865.576.5728
Email: reports@osti.gov

Available for sale to the public, in paper, from:

U.S. Department of Commerce
National Technical Information Service
5301 Shawnee Road
Alexandria, VA 22312
NTIS http://www.ntis.gov
Phone: 800.553.6847 or 703.605.6000
Fax: 703.605.6900
Email: orders@ntis.gov

Cover Photos by Dennis Schroeder: (left to right) NREL 26173, NREL 18302, NREL 19758, NREL 29642, NREL 19795.

NREL prints on paper that contains recycled content.
DISCLAIMER

The Standard Bio-oil Analytical Methods (Methods) are provided by the National Renewable Energy Laboratory (NREL), which is operated by Alliance for Sustainable Energy, LLC (Alliance) for the U.S. Department of Energy (DOE). These methods were developed and written for commercial research and educational use only.

Access to and use of these Methods shall impose the following obligations on the user. The user is granted the right, without any fee or cost, to use, copy, modify, alter, enhance and distribute these Methods for any purpose whatsoever, except commercial sales, provided that this entire notice appears in all copies of the Methods. The user agrees to credit NREL/Alliance in any publications that result from the use of these Methods. The user also understands that NREL/Alliance is not obligated to provide the user with any support, consulting, training or assistance of any kind with regard to the use of these Methods or to provide the user with any updates, revisions or new versions.

THESE METHODS ARE PROVIDED BY NREL/Alliance "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL NREL/Alliance/DOE BE LIABLE FOR ANY SPECIAL, INDIRECT OR CONSEQUENTIAL DAMAGES OR ANY DAMAGES WHATSOEVER, INCLUDING BUT NOT LIMITED TO CLAIMS ASSOCIATED WITH THE LOSS OF DATA OR PROFITS, WHICH MAY RESULT FROM AN ACTION IN CONTRACT, NEGLIGENCE OR OTHER TORTIOUS CLAIM THAT ARISES OUT OF OR IN CONNECTION WITH THE ACCESS, USE OR PERFORMANCE OF THESE METHODS.
1. **Introduction**

1.1 It is necessary to quantify the amount of hydroxyl groups present in pyrolysis bio-oils. Hydroxyl groups (-OH) are typically derived from carbohydrate fragments as well as products of reduction/hydrogenation of carbonyls. Together with other techniques (e.g. carbonyl titration, acid titration), hydroxyl analysis can give a complete picture of the different types of oxygen functionalities present in bio-oils.

1.2 $^{31}$P NMR (nuclear magnetic resonance) has previously been used to measure -OH present in coal and lignin. Similar to $^1$H, the natural abundance of $^{31}$P nuclei is 100%, making it an attractive nucleus to track. During sample preparation, the bond between the P and Cl in the phosphitylating agent hydrolyzes, allowing the alkoxide, phenoxide or carboxylate to react with the phospholane. The main reaction is illustrated in the reaction scheme below:

$$
\begin{align*}
\text{O} & \quad \text{P} - \text{Cl} + R - \text{OH} + \text{NEt}_3 \\
\rightarrow & \\
\text{O} & \quad \text{P} - \text{O} \quad \text{R} + [\text{HNEt}_3]\text{Cl}
\end{align*}
$$

1.3 This procedure covers the determination of -OH in aliphatic, phenolic and carboxylic acid groups. The results are reported as mmol OH per gram of bio-oil. Alternatively, results can be reported as gram O per gram of bio-oil. A recent publication provides some background on the use of $^{31}$P NMR for analysis of bio-oils [1].

2. **Scope**

2.1 This procedure has been optimized for the quantification of hydroxyls (-OH) in bio-oil from aliphatics, phenolics and carboxylic acids using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as the phosphitylating agent and triphenylphosphine oxide (TPPO) as the internal standard. This procedure was developed and validated for the analysis of raw pyrolysis bio-oil.

2.2 The amount of water in the sample is taken into consideration in calculating the amount of TMDP needed during sample preparation. Water content can be reliably quantified in bio-oils using Karl Fischer titration [2].

2.3 The O content of the bio-oil sample is needed in the calculation to account for the required amount of TMDP. The O content of bio-oils can be reliably quantified by elemental analysis.

3. **Terminology**

3.1 *Bio-oil* – The crude liquid product of converting lignocellulosic biomass into a liquid via fast pyrolysis or other thermochemical conversion process.
3.2  *Phosphitylation* - reaction wherein the labile H in the -OH groups is exchanged with the P-containing agent in the presence of a suitable solvent system.

3.3  *Relaxation delay time* - the time required to allow for full relaxation of the excited nuclei back to its ground state.

### 4. Interferences

4.1  Water reacts with the phosphitylating agent TMDP. Reacted TMDP has yellow precipitates in the reagent bottle.

4.2  The pyridine:water ratio is critical in maintaining a one-phase NMR solution. A minimum ratio of 185 (mass pyridine: mass water) was found to be sufficient.

4.3  Amines can interfere in the quantification of the hydroxyls [3]. The effect of this will be further studied.

### 5. Apparatus

5.1  Analytical balance, accurate to 0.1 mg

5.2  Schlenk line or nitrogen gas source

5.3  NMR Instrument, a 500 mHz unit is recommended

### 6. Reagents and Materials Needed

6.1  Reagents

6.1.1  Chromium acetylacetonate, reagent/analytical grade (Cr(acac)_3)

6.1.2  Triphenylphosphine oxide, reagent/analytical grade (TPPO)

6.1.3  Pyridine, anhydrous

6.1.4  Chloroform, deuterated (CDCl₃)

6.1.5  2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, reagent grade (TMDP)

6.2  Materials

6.2.1  Activated molecular sieves (for moisture removal)

6.2.2  Scintillation vials (20 ml)

6.2.3  Syringes (~1 ml)

6.2.4  Needles
6.2.5 Spatula
6.2.6 Glass transfer pipettes
6.2.7 NMR tubes

7. ES&H Considerations and Hazards

7.1 Do not inhale or make contact with TMDP.
7.2 Pyridine is harmful if inhaled, swallowed or absorbed through the skin. It causes serious eye irritation.
7.3 Follow all applicable chemical handling procedures.

8. Sampling, Test Specimens and Test Units

8.1 Bio-oil should be allowed to equilibrate to room temperature for at least 20 minutes before sampling.
8.2 Care must be taken to ensure that a representative sample is taken for analysis. Shake bio-oil at ambient temperature as vigorously as possible.
8.3 It is best to use the TMDP inside a glove box. However, in the absence of one, contamination can be minimized by using small volume reagent containers (i.e. 1 g). This will allow for about 3-4 NMR samples to be prepared and thus limits the amount of opening of closing of the vessel.

9. Analytical Procedure

9.1 Determination of oxygen and moisture content.
9.1.1 The oxygen and moisture content of the bio-oil sample must be determined before running this $^{31}$P NMR method.
9.1.2 Oxygen content for high-water containing bio-oils is typically determined by difference from the result of C,H,N,S elemental analysis ([4] and [5] for CHN, [6] and [7] for S). If the sample has low water content (less than 5%), oxygen content can be directly determined by ASTM D5373 [5]. If the laboratory does not have this capability, external analytical laboratories can be used for these analyses.
9.1.3 Karl-Fisher titration method, ASTM D4928-12 [2], is the recommended method for determining water content in bio-oil. If the laboratory does not have this capability, external analytical laboratories can be used.
9.2 Preparation of solvent mixture.

9.2.1 Minimize moisture uptake of the deuterated solvent, CDCl$_3$, by adding activated molecular sieves into the reagent bottle after opening.

9.2.2 Dispensing aliquots from the anhydrous pyridine reagent bottle needs to be done under inert atmosphere (e.g. N$_2$). Attach a needle to a Schlenk line or regulated low pressure N$_2$ source. Insert this needle and another needle, as outlet, into the septum, to keep the space above the liquid filled with the inert gas.

9.2.3 For preparation of multiple samples to be analyzed in succession, a solvent mixture with the internal standard can be prepared. Volumes are quoted for easy measurement and dispensing but weights of every chemical need to be noted. Outlined below are amounts for sufficient preparation for 2 samples (with excess that can be used for 1 more, if needed). Important reagent amount relationships are also summarized in the following table:

<table>
<thead>
<tr>
<th>Pyridine:CDCl$_3$</th>
<th>1.6:1</th>
<th>mL pyridine: mL CDCl$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(acac)$_3$ concentration</td>
<td>0.003</td>
<td>mmol Cr(acac)$_3$/mL solvent solution</td>
</tr>
<tr>
<td>TPPO concentration</td>
<td>0.025</td>
<td>mmol TPPO/mL solvent</td>
</tr>
<tr>
<td>Minimum pyridine:water ratio</td>
<td>185</td>
<td>g pyridine/g water</td>
</tr>
</tbody>
</table>

9.2.3.1 Measure 2.31 ml of CDCl$_3$ into a pre-weighed scintillation vial. Record CDCl$_3$ weight.

9.2.3.2 Add about 6.3 mg of the relaxant, Cr(acac)$_3$. Record actual weight. Shake to dissolve in the solvent.

9.2.3.3 Add about 41.7 mg TPPO. Record actual weight. Shake to dissolve in the solvent.

9.2.3.4 This mixture will be referred to as the ISTD solution.

9.2.4 For preparation of one NMR sample using the prepared ISTD solution:

9.2.4.1 Transfer 0.6 ml of the ISTD solution into a pre-weighed scintillation vial. Record actual weight.

9.2.4.2 Transfer 0.9 mL of anhydrous pyridine into the vial. Record actual weight.

9.2.4.3 Measure about 18 mg of bio-oil into the vial. Record actual weight.
9.2.4.4 Add TMDP in an amount equivalent to 2mmol/mmol H₂O + 1mmol/mmol O content. For raw pyrolysis bio-oils, this tends to be ~210 mg of TMDP. Record actual weight.

9.2.4.5 Shake mixture and confirm that there are no precipitates. In the event that precipitates do appear, pyridine and CDCl₃ will need to be added at 1.6 pyridine:CDCl₃ ratio until the precipitate is no longer visible.

Note: A pyridine:water (mass pyridine:mass water) ratio of 185 and higher was found to be sufficient to prevent precipitation from occurring.

9.2.4.6 Transfer solution into an appropriate NMR tube.

9.3 NMR experiment

9.3.1 Check the NMR instrument and make sure that the correct probe for phosphorous detection is in place.

9.3.2 Put the sample inside the NMR.

9.3.3 Adjust parameters: lock, shimming and pulse program. The following are the NMR parameters that will be used.

<table>
<thead>
<tr>
<th>NMR parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of scans</td>
<td>Greater than 128 scans</td>
</tr>
<tr>
<td>Pulse width</td>
<td>90°</td>
</tr>
<tr>
<td>Acquisition time</td>
<td>1.2 sec</td>
</tr>
<tr>
<td>d1</td>
<td>25 sec</td>
</tr>
<tr>
<td>Decoupling</td>
<td>Inverse-gated</td>
</tr>
</tbody>
</table>

9.3.4 Collect spectra.

9.4 Data analysis with MestreNova software

9.4.1 Apodize the file by setting line broadening to exponential and value of 5 Hz.

9.4.2 Adjust phase.

9.4.3 Reference spectra by assigning the TMDP peak at 175.514 ppm.

9.4.4 Make this peak as symmetrical as possible through zero order phasing (PH0).

9.4.5 Look for the TPPO peak and its satellites (between 27 and 28 ppm).
9.4.6 Adjust first order phasing (PH1) to make the TPPO (and the other peaks) as symmetrical as possible.

9.4.7 Take note of the range of the TPPO peak and its satellites.

9.4.8 Adjust baseline. Bernstein polynomial fit, parameter = 6 is typically used.

9.4.9 Integrate peak regions.

9.4.9.1 Make sure that the calculation method used is "Sum" method.

9.4.9.2 The regions of peaks used in the measurement are as follows:

- TMDP: peak assigned at 175.514 ppm
- 145.0 - 152.0 ppm - aliphatic OH
- 138.0 - 145.0 ppm - phenolic OH
- 134.6 - 138.0 ppm - carboxylic acid OH
- 130.0 - 133.7 ppm - water adduct (di-phosphitylated)

TPPO range: 27-29 ppm. Adjustment needed to include satellites on other side of main peak

9.4.10 Calculate the ratio of the different peaks with respect to the TPPO peak.

9.4.11 Calculate the amount of the TPPO in the sample. The amount of each region will be calculated based on the TPPO amount.

10. Results

10.1 The following tables can be used as a guide to record data.

10.1.1 Preparation of the ISTD solution:

<table>
<thead>
<tr>
<th>TPPO purity, %</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCl₃</td>
<td></td>
</tr>
<tr>
<td>Cr(acac)₃</td>
<td></td>
</tr>
<tr>
<td>TPPO</td>
<td></td>
</tr>
<tr>
<td>Total Mass ISTD</td>
<td></td>
</tr>
</tbody>
</table>
10.1.2 Preparation of NMR sample:

<table>
<thead>
<tr>
<th></th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISTD solution</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td></td>
</tr>
<tr>
<td>Bio-oil</td>
<td></td>
</tr>
<tr>
<td>TMDP</td>
<td></td>
</tr>
<tr>
<td>Total Mass NMR Sample</td>
<td></td>
</tr>
</tbody>
</table>

11. Calculations

11.1 Calculate the TPPO concentration in NMR sample, \([TPPO]\)

\[
[TPPO] = \frac{\text{mmol TPPO}}{\text{mass (g) of NMR sample}} = \frac{\text{total mass (g) ISTD solution} \times \text{mass (g) of ISTD solution in NMR sample}}{\text{total mass (g) NMR sample}}
\]

where:

\[
\text{mmol TPPO} = \frac{\text{mass (g) of TPPO} \times \text{TPPO purity}}{278.29 \text{ g mol}^{-1} \text{ TPPO}} \times \frac{100}{1000}
\]

11.2 Calculate the ratio of spectral region \(i\) over TPPO, \(I_i/I_{TPPO}\)

\[
\frac{I_i}{I_{TPPO}} = \frac{\text{integration of spectral region of interest}}{\text{integration of TPPO region}}
\]

where: \(i = \) aliphatic, phenolic or carboxylic region

11.3 Calculate the amount of hydroxyl in region \(i\) in bio-oil, mmol OH\(_i\)/g bio-oil

\[
\frac{\text{mmol OH}_i}{g \text{ biooil}} = \frac{I_i}{I_{TPPO}} \times [TPPO] \times \text{total mass (g) of NMR sample} \times \text{mass (g) bio-oil}
\]

where: \(i = \) aliphatic, phenolic or carboxylic region

11.4 Calculate the amount of O associated with region \(i\) in bio-oil, g O\(_i\)/g bio-oil

11.4.1 Aliphatic or phenolic O:

\[
\frac{g \text{ O}_{\text{aliphatic/phenolic}}}{g \text{ biooil}} = \frac{\text{mmol OH}_{\text{aliphatic/phenolic}}}{g \text{ biooil}} \times \frac{16 \text{ mg O}}{\text{mmol}} \times \frac{g}{1000 \text{ mg}}
\]
11.4.2 Carboxylic O:

\[
g \frac{O_{\text{carboxylic}}}{g \text{ biooil}} = \frac{mmol \text{ OH}_{\text{carboxylic}}}{g \text{ biooil}} \times \frac{2 \text{ mol O}}{\text{mol OH}} \times \frac{16 \text{ mg O}}{\text{mmol}} \times \frac{g}{1000 \text{ mg}}
\]

12. Report Format

12.1 Report the average amount of hydroxyl per region in bio-oil as mmol OH per gram of bio-oil (11.3). Alternatively, results can be reported as g O per gram of bio-oil (11.4). Standard deviation may also be reported.

13. Precision and Bias

13.1 In 2015, an inter-laboratory study was performed on a raw pyrolysis bio-oil using the method as described here [8]. An NMR technique has never been tested in an inter-laboratory study on bio-oil analysis, and the $^{31}$P NMR technique here produced acceptable variabilities among the labs. With inter-laboratory variabilities less than 10% RSD, aliphatic and phenolic OH groups can be reliably quantified using this method, but carboxylic OH groups were prone to larger variabilities, on the order of 15%.

14. Quality Control

14.1 Reported Significant Figures: Report results with two decimal places.

14.2 Replicates: Run all samples in triplicate.

15. References


