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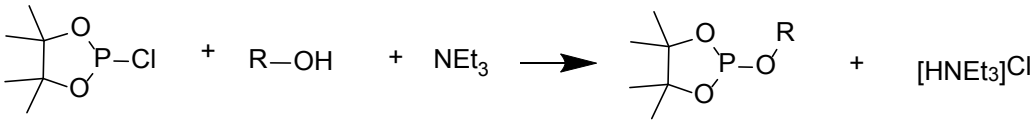
1. Introduction

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:



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 ($CDCl_3$)

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:

Pyridine:$CDCl_3$	1.6:1	mL pyridine: mL $CDCl_3$
$Cr(acac)_3$ concentration	0.003	mmol $Cr(acac)_3$ /mL solvent solution
TPPO concentration	0.025	mmol TPPO/mL solvent
Minimum pyridine:water ratio	185	g pyridine/g water

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 H_2O + 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 $CDCl_3$ will need to be added at 1.6 pyridine:$CDCl_3$ 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.

NMR parameters	
Number of scans	Greater than 128 scans
Pulse width	90°
Acquisition time	1.2 sec
d1	25 sec
Decoupling	Inverse-gated

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-phosphytilated)

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:

TPPO purity, %	
	Mass (g)
$CDCl_3$	
$Cr(acac)_3$	
TPPO	
Total Mass ISTD	

10.1.2 Preparation of NMR sample:

	Mass (g)
ISTD solution	
Pyridine	
Bio-oil	
TMDP	
Total Mass NMR Sample	

11. Calculations

11.1 Calculate the TPPO concentration in NMR sample, [TPPO]

$$[TPPO\,] = \frac{mmol\ TPPO}{mass\ (g)\ of\ NMR\ sample}$$

$$= \frac{\left(\frac{mmol\ TPPO}{total\ mass\ (g) ISTD\ solution}\right) x\ mass\ (g) of\ ISTD\ solution\ in\ NMR\ sample}{total\ mass\ (g)\ NMR\ sample}$$

where:

$$mmol\ TPPO = \frac{mass\ (g)\ of\ TPPO}{278.29 \frac{g}{mol} TPPO}\ x\ \frac{TPPO\ purity}{100}\ x\ 1000$$

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

$$\frac{I_i}{I_{TPPO}} = \frac{integration\ of\ spectral\ region\ of\ interest}{integration\ of\ TPPO\ region}$$

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

$$\frac{mmol\ OH_i}{g\ biooil} = \frac{\frac{I_i}{I_{TPPO}} x\ [TPPO] x\ total\ mass\ (g)\ of\ NMR\ sample}{mass\ (g)\ bio-oil}$$

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:

$$\frac{g\ O_{aliphatic/phenolic}}{g\ biooil} = \frac{mmol\ OH_{aliphatic/phenolic}}{g\ biooil}\ x\ \frac{16\ mg\ O}{mmol}\ x\ \frac{g}{1000\ mg}$$

11.4.2 Carboxylic O:

$$\frac{g\ O_{carboxylic}}{g\ biooil} = \frac{mmol\ OH_{carboxylic}}{g\ biooil}\ x\ \frac{2\ mol\ O}{mol\ OH}\ x\ \frac{16\ mg\ O}{mmol}\ x\ \frac{g}{1000\ mg}$$

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

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