

SP101 107

EVALUATION OF MAGNETIC FIELD EFFECT ON QUANTIFICATION OF AROMATIC COMPONENTS OF HUMIC ACIDS BY ¹³C CPMAS NMR

Martha González Pérez^{1,2(*)}, Cristina Santín Nuno³, Luiz Alberto Colnago², Heike Knicker⁴,
Pablo Vidal Torrado¹, Ladislau Martin Neto²

1. Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ-USP).

Departamento de Solos e Nutrição de Plantas. C.P. 09, CEP 13418-900. Piracicaba-SP, Brazil.

2. Embrapa Instrumentação Agropecuária. C.P.741, CEP: 13560-970. São Carlos, SP, Brazil.

3. INDUROT, Universidad de Oviedo, Campus de Mieres, 33600 Mieres, Asturias, Spain.

4. Technische Universität München, Department of Soil Science, 85350. Freising-Weihenstephan, Germany.

(*) e-mail: marta@cnpdia.embrapa.br

Keywords: soil humic acids; NMR; VACP/MAS

High resolution ¹³C NMR spectroscopy, acquired with the CPMAS technique, has been a powerful tool to analyze chemical composition of soil organic matter (SOM), since 1980¹. The principal application of the CPMAS technique has been the quantification of the structural components of humic substances such as the amount of aromatic, aliphatic, carbonyl and carboxyl groups. As in all NMR areas, the use of high magnetic field spectrometers to SOM analysis has become popular. However, the uses of high magnetic field spectrometers (HMF) have introduced some undesirable effects. One of these effects, caused by MAS, has been the cross-polarization profile breakage down. As the speed of MAS increase with the magnetic field, the oscillatory behavior of conventional CP became worse and consequently the quantification of the NMR signals. For this reason it is not possible to compare the SOM group quantification obtained by CPMAS in different magnetic field spectrometers. To minimize this problem, Peerson introduced in 1993² the variable amplitude cross polarization (VACP) procedure that reduces oscillatory behavior of CP. This technique has been used in most SOM NMR analysis up to 9.4 T, but as we are showing in this paper it is not enough to solve the problem in higher field such as 17.6T (750 MHz for 1H). The aim of the present work is to show the poor efficiency of VACP pulse sequence in high magnetic field.

The soil samples were collected from a reclaimed estuary in Asturias, Spain. It was selected a profile under pasture. The samples were collected at 0-20 cm depth (Pa), 40-60 cm depth (Pb) and more than 100 cm depth (Pc). Humic acids were extracted using the IHSS method (extraction with 0.1 mol L⁻¹ NaOH and precipitation with 6 mol L⁻¹ HCl).

¹³C VACP/MAS NMR spectra were acquired with a Bruker DSX 200 spectrometer at the ¹³C resonance frequency of 50.32 MHz. The samples were confined in a zirconium oxide rotor. The variable amplitude cross polarization magic angle spinning technique was applied with a contact time of 1 ms, a spinning speed of 7 kHz and a pulse delay of 1 s. The spectra were subdivided into different chemical shift regions: alkyl C (0-45 ppm), N-alkyl/methoxyl C (45-60 ppm), O-alkyl C (60-110 ppm), aromatic C (110-160 ppm), carboxyl C (160-185 ppm) and carbonyl-C (185-245 ppm). The relative ¹³C intensity distribution was determined by integrating the signal intensity in different chemical shift regions with an integration routine supplied with the instrument software. The same technique and parameters were used on a Varian Unity 750 spectrometer at the ¹³C resonance frequency of 188.55 MHz.

The figure 1 and figure 2 show the spectra measured on a 5 and 17.6 T spectrometers, respectively. The shape and the intensity of resonance lines from aliphatic region from 0 to 110 ppm are similar in both spectrometers. However, resonance lines from aromatic region from 110 to 165 ppm are very weak on spectra measured at 17.6 T. Most of the signals in this region is associated to the side band of the carboxyl signals at 183 ppm.

The real aromatic region signal obtained at 17.6 T is shown in figure 3, after subtracting the side bands. This result indicates the difficulties involved in the quantification of aromatic groups at HMF and consequently the comparison with published results.

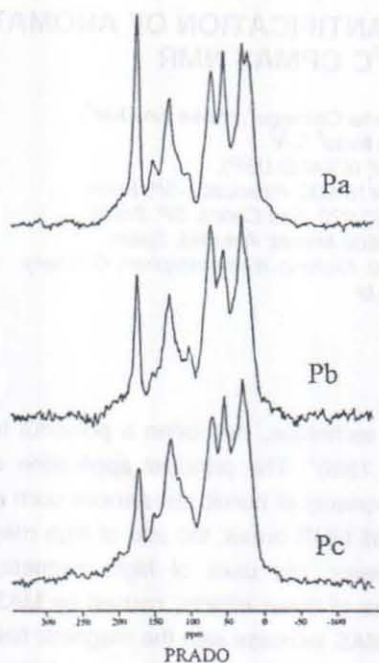


Figure 1. ^{13}C NMR VACP MAS spectra measured on a Bruker 200 MHz spectrometer.

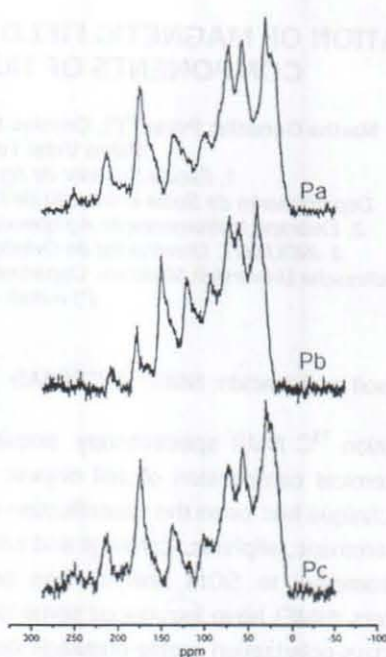


Figure 2. ^{13}C NMR VACP MAS spectra measured on a Varian 750 MHz spectrometer.

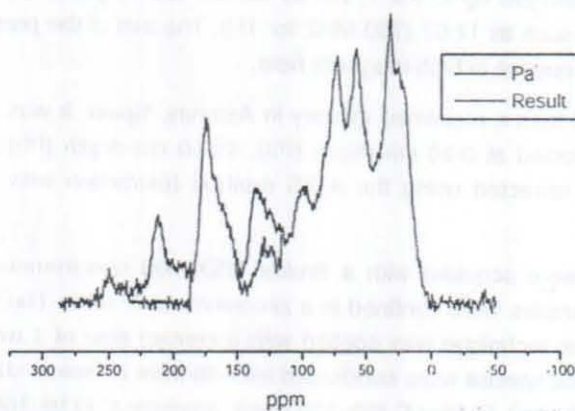


Figure 3. ^{13}C NMR VACP MAS spectra measured on a Varian 750 MHz spectrometer after subtracting the strong side bands.

Our results show the poor efficiency of VACP pulse sequence in high magnetic field demonstrated by the dramatic suppression of aromatic signals from humic acids. Thus, the recommendation for SOM NMR researchers is to be careful with the utilization of HMF because the VACP technique has not been efficient enough to minimize the oscillatory behavior of conventional CP and enhance the quality of spectra.

REFERENCES

1. Wilson, M.A., 1987. NMR Techniques and Application. In Geochemistry and Soil Chemistry. Pergamon Press, Oxford.
2. Peersen, O.B.; Xiaoling, W.; Kustanovich, I.; Smith, S.O. *J Mag Res. A* 1993, 104, 334.