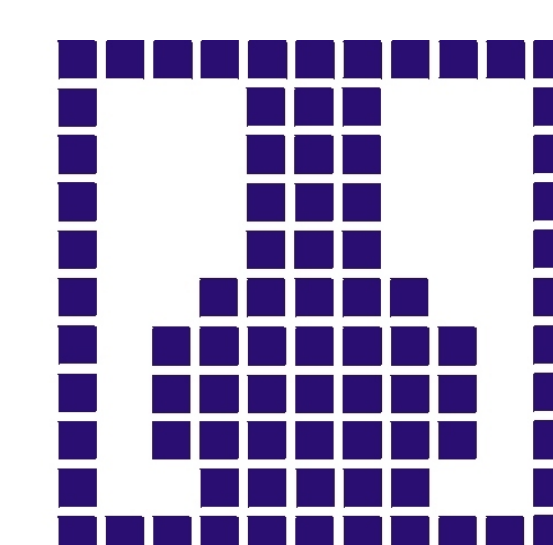




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IMPROVED METHODS for the DETERMINATION of HYDROXYPROPYLATION in MODIFIED STARCH USING NMR and COMPARISON with CLASSICAL METHODS



CEQUIPE

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Abstract / Resumen

Improved methods using high-resolution NMR were evaluated as rapid measurements for determining the hydroxypropyl content of modified starch.

Six samples of modified starch were measured and the results were compared with the results of other known methods like IR, colourimetric and the Food-Chemical-Codex.

The different advantages and disadvantages of the mentioned methods were elaborated and

Métodos optimizados probados usando RMN de alta resolución fueron evaluados para una medida rápida en la determinación del contenido de grupos hidroxipropilo en almidones modificados.

Seis muestras de almidones modificados fueron analizadas y los resultados obtenidos fueron comparados con los resultados conocidos de otros métodos tales como IR, métodos colorimétricos y métodos del Food-Chemical-Codex.

Introduction

Large quantities of starch are chemically and/or physically modified to obtain desired properties for different applications. One important product group is hydroxypropylated starch that has a wide spectrum of application areas, mainly in food and food-related products. Use of hydroxypropylated starch gives improved shelf life, freeze/thaw stability, cold storage stability, cold water swelling and reconstituting properties to a formulated product.

It is essential to study the relationships between modification procedure, functional properties and structure to enable control of the derivatisation process. Therefore, it is of great importance to reliably determine parameters like the hydroxypropyl level (% HP) in modified starches.

Several methods have been reported, including a colourimetric method, IR and NMR.

Materials and Methods

NMR measurements

NMR in Dimethylsulfoxid:

10 mg of modified starch (not dried) + 1 ml of DMSO-d₆ in 25 ml test-tube placed in a boiling-water bath for 5-10 min cooling down to room-temperature + 30 mg of solution of acetic acid (10-20 mg in 1 ml D₂O) as internal standard 0.5 ml of suspension transferred into NMR-tube shaken / not shaken (see Figure 5) spectras acquired on 400 MHz-BRUKER-UltraShield-NMR-Spectrometer see Figure 1

NMR in hydrochloric acid:

50 mg modified starch + 1 ml 10 % HCl (conc. HCl with D₂O) solution heated in water bath for 15 minutes + cooling down + internal standard (acetic acid, 50 mg in 1 ml D₂O) measurement see above (Figure 1)

NMR in trifluor-acetic acid:

Instead of 10 % HCl (conc. HCl with D₂O) 20 % solution of trifluor-acetic acid (100 % for spectroscopy in D₂O) was used

IR measurements

0.25 mg of starch + 0.5 mg of KBr mixed, triturated and transferred to a die for pressing

die evacuated for 5 min pressed under a load of 100 kg/cm² for 5 min, then 200 kg/cm² for 10 min spectra acquired using a NICOLET-IMPACT 400D (32 scans, background-corrected) see Figures 2 and 4

Colourimetric method

spectrophotometric determination of hydroxypropyl-group was carried out using the method of Johnson (see References)

method involves hydrolysis of the group to propylene glycol which in turn (concentrated sulfuric acid) is dehydrated to propionaldehyde and the enolic form of allyl alcohol

products are measured spectrophotometrically after reaction with ninhydrin (purple color) see Figure 3

Food-Chemical-Codex

method was performed using the Food-Chemical-Codex (see References)

Figure 1 (NMR):

The % HP was calculated using the integrals of the signal of the methyl-protons of the acetic acid (around 1.5 ppm) and the signal of the methyl-protons of the hydroxypropyl-group (around 0.5 ppm).

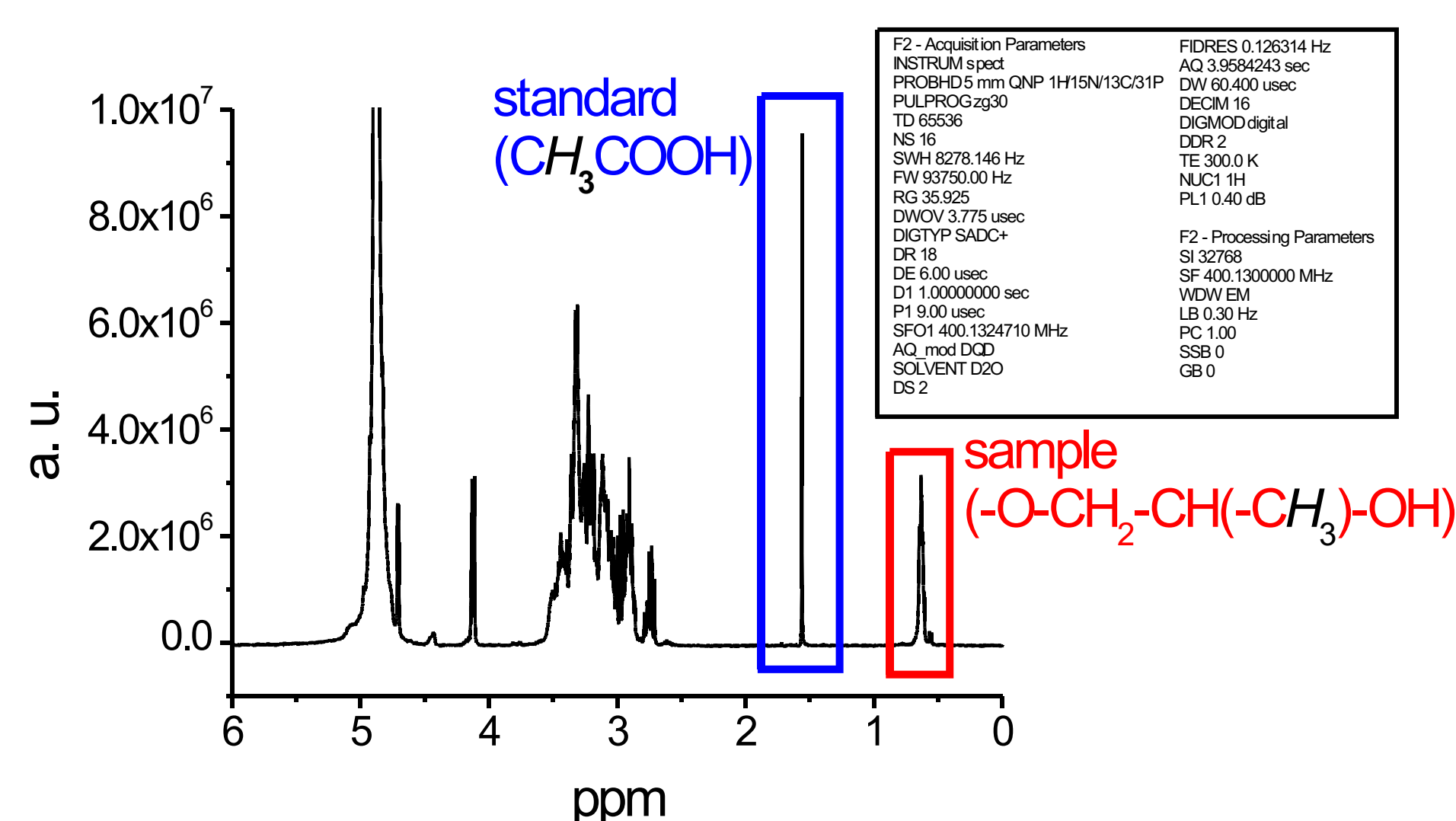


Figure 2 (IR):

The shoulder at 2974 cm⁻¹ (C-H stretch of the methyl-group) is characteristic for the hydroxypropyl-group.

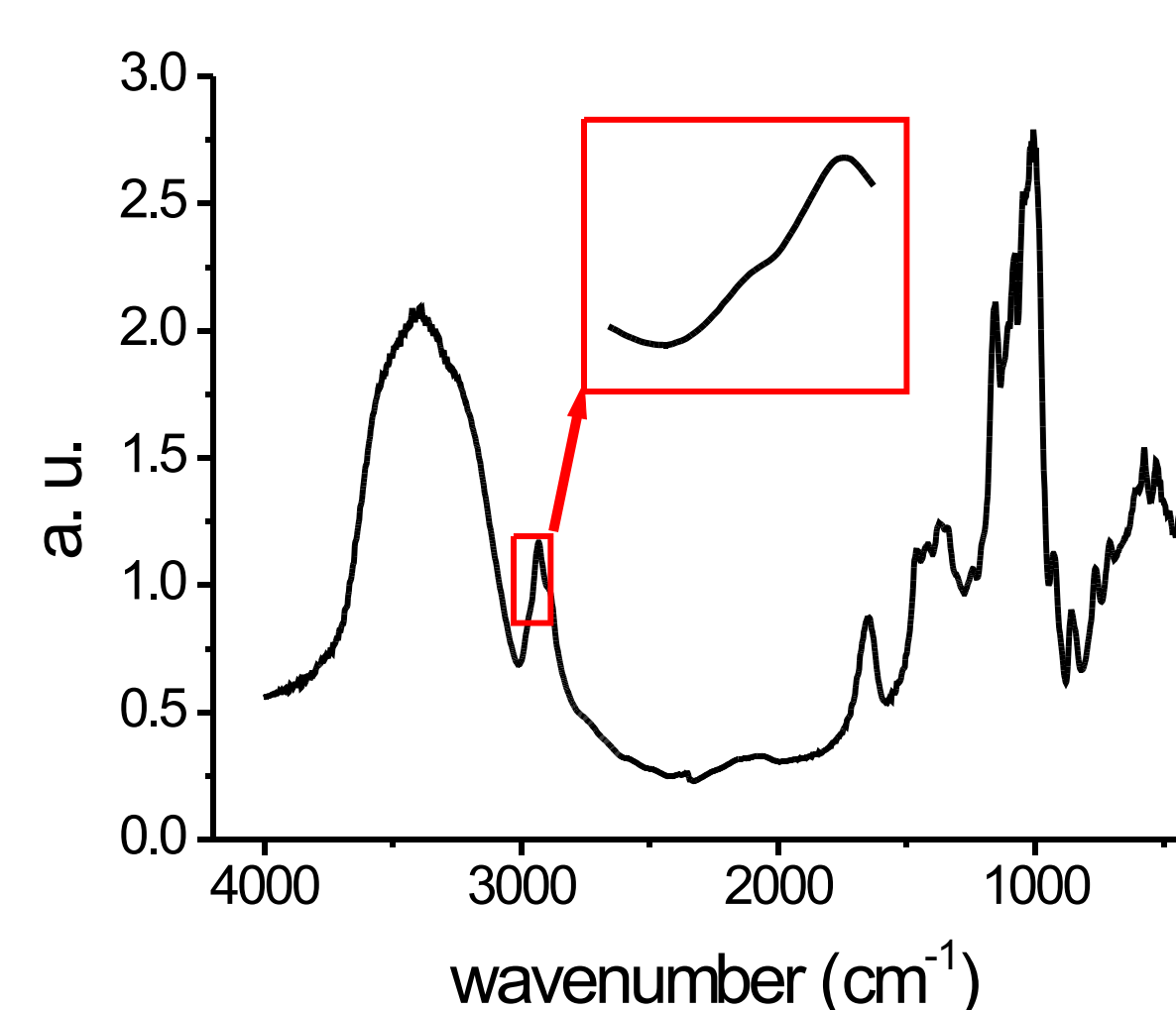


Figure 4 (IR):

The average of 2-4 spectras were two times automatically smoothed and the second derivative of the absorbance was calculated. The area of the peak centered at 2974 cm⁻¹ minus the area of a "blank" (unmodified starch) is proportional to the amount of hydroxypropylation (areas obtained by drawing a horizontal baseline, see Figure).

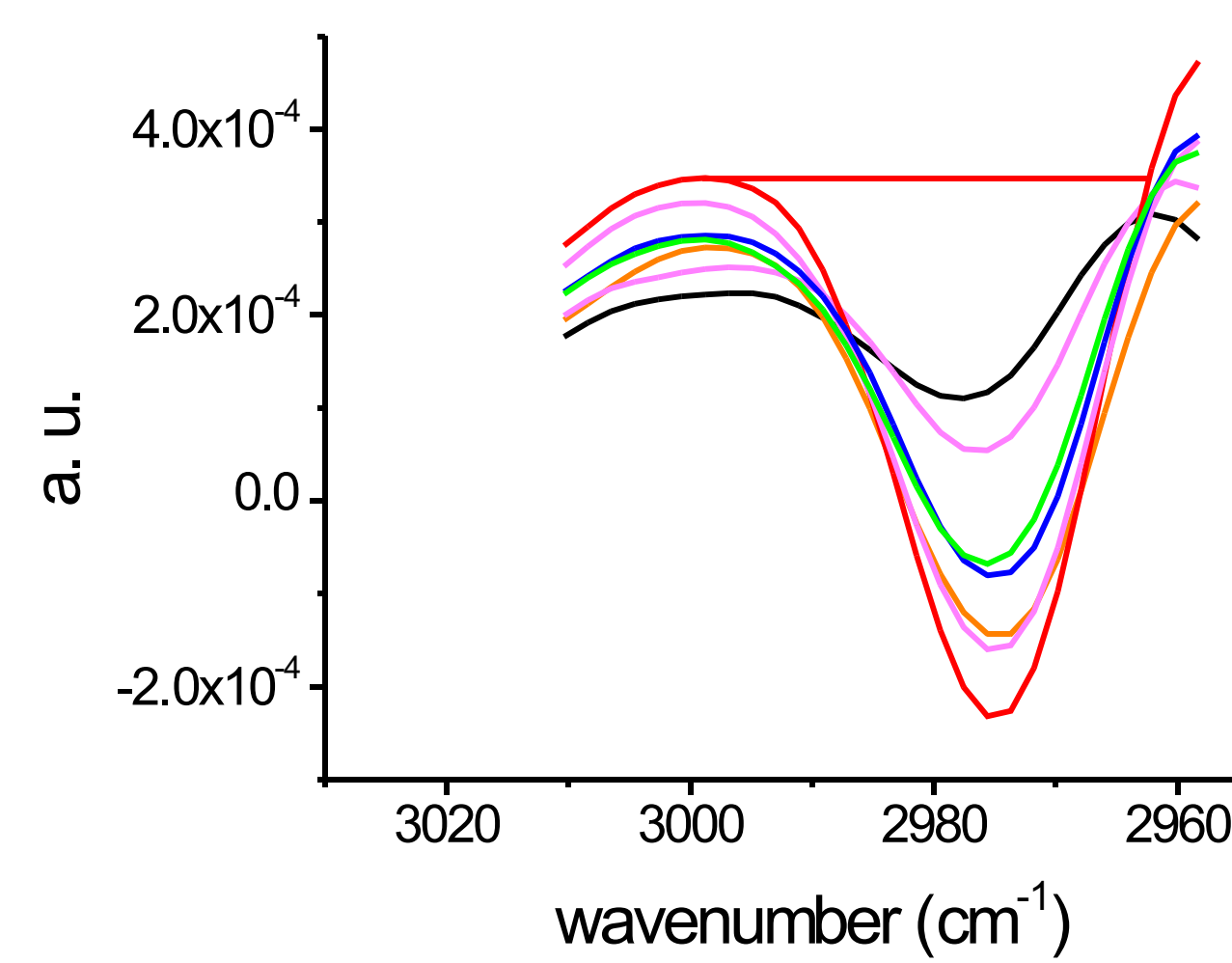


Figure 3 (colourimetric):

The figure shows the spectra of the resulted product (ninhydrin reaction) of the Johnson (References) method. The maximum is clearly visible at 590 nm and the absorbance is proportional to the amount of hydroxypropylation.

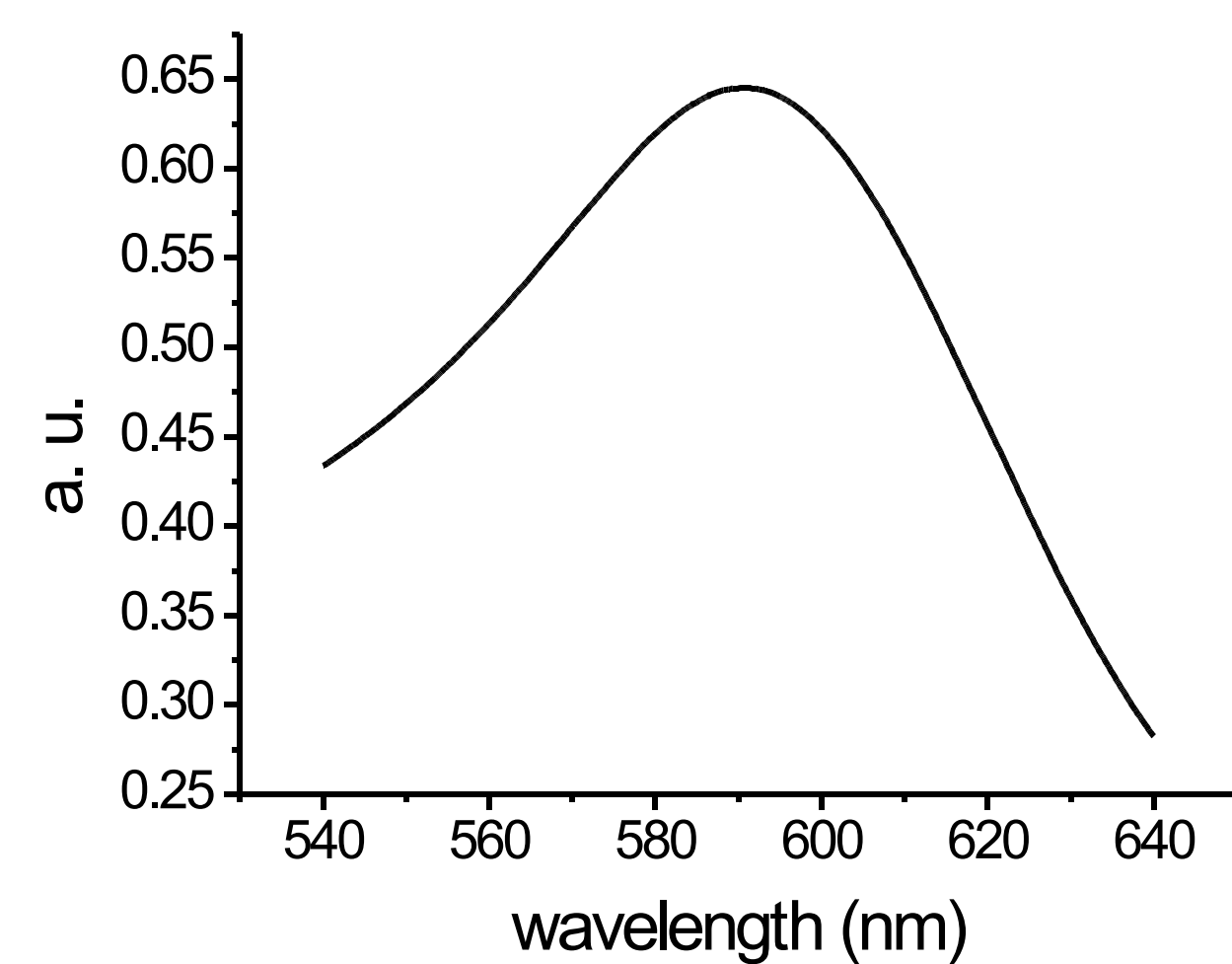


Figure 8:

This figure shows good agreement between the methods using F₃CCOOH and HCl, although the % HP values obtained with HCl were slightly higher (reason unknown).

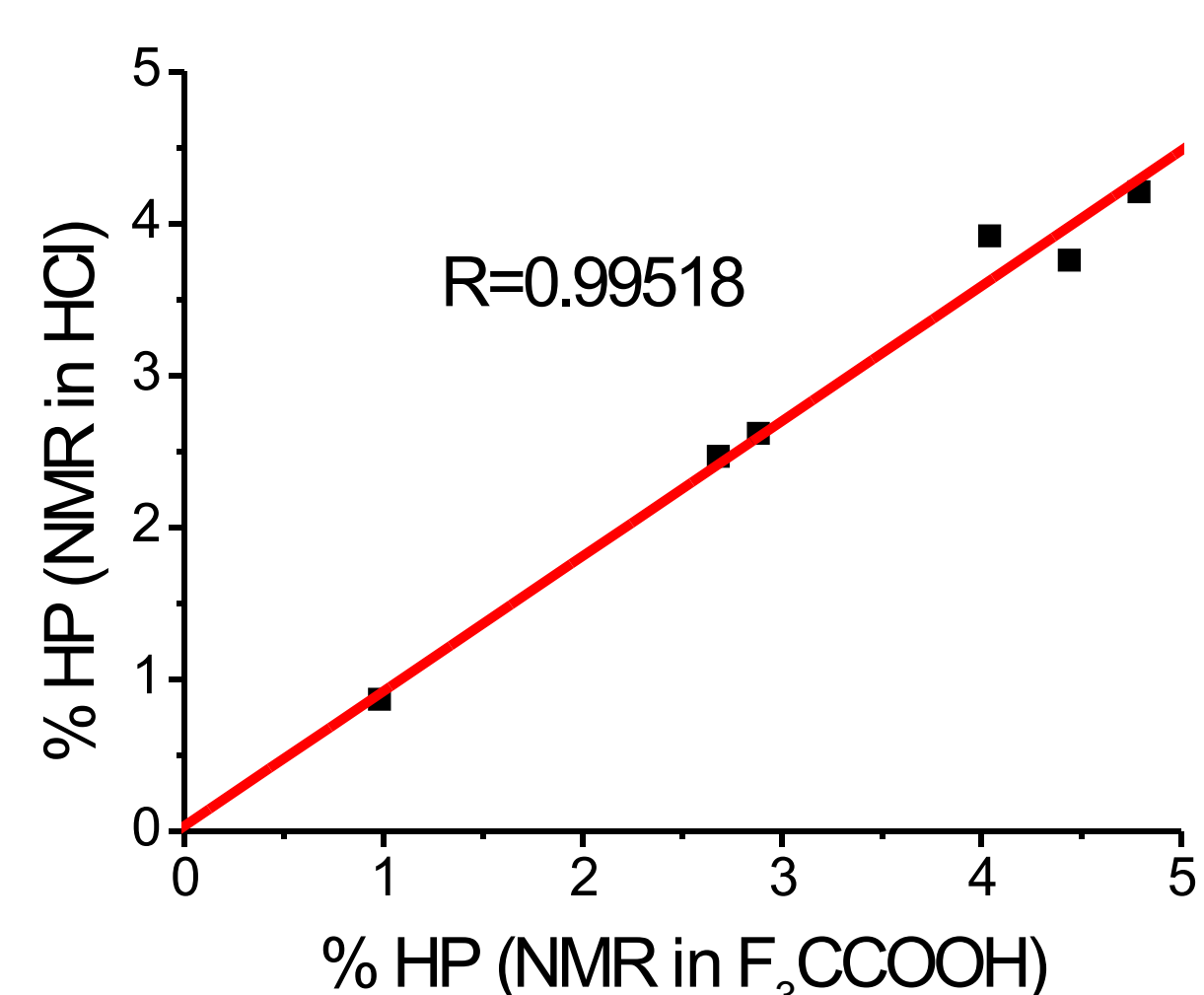


Figure 9:

The plot (see Figure) shows good agreement between the NMR and the IR method. Note that the IR method delivers areas only.

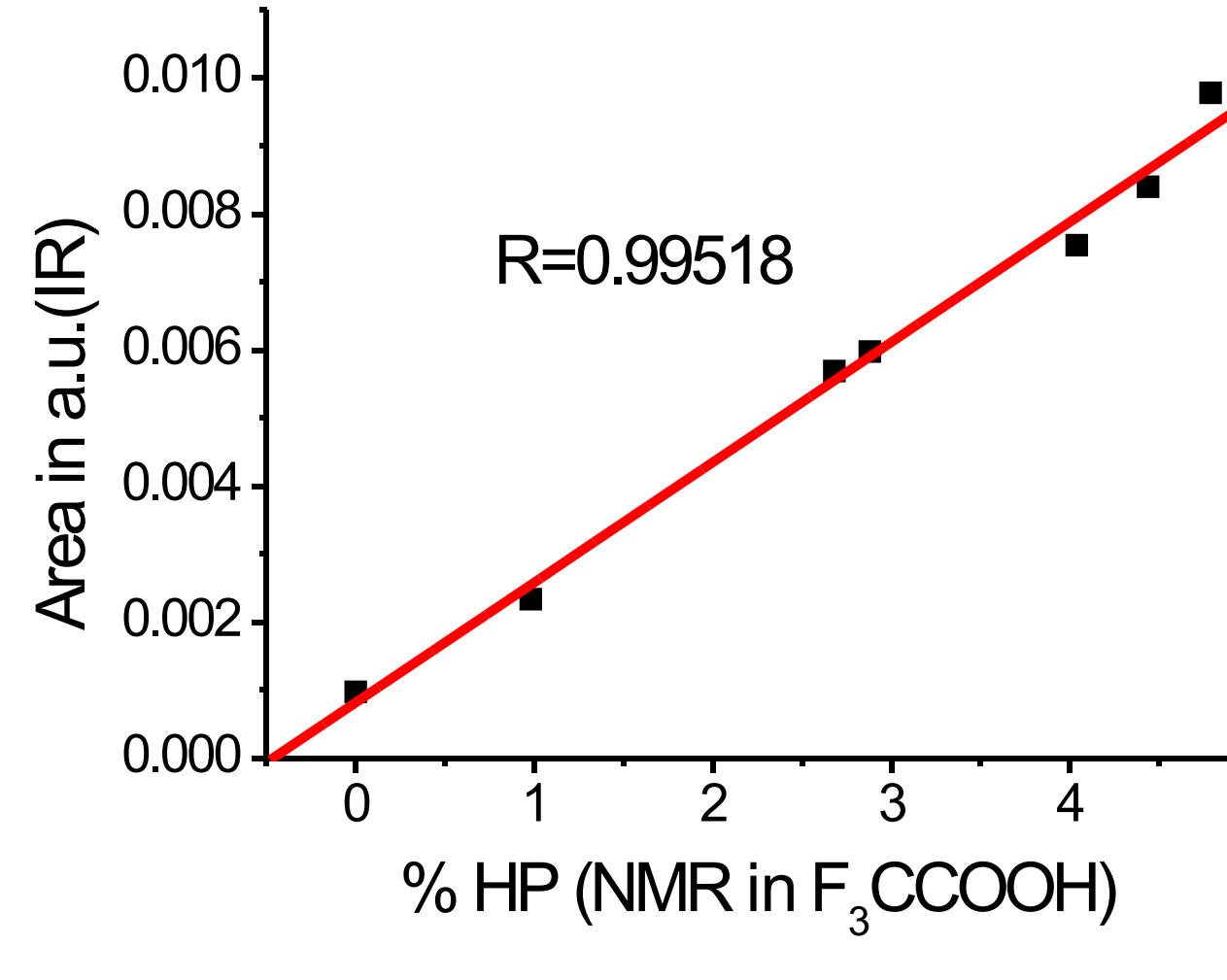


Figure 5:

The differences (*) are all significant at a level of 0.01 (t-test). Therefore the DMSO method is an approximate method and it is strongly recommended to shake the NMR-tubes well when using DMSO-d₆ as solvent.

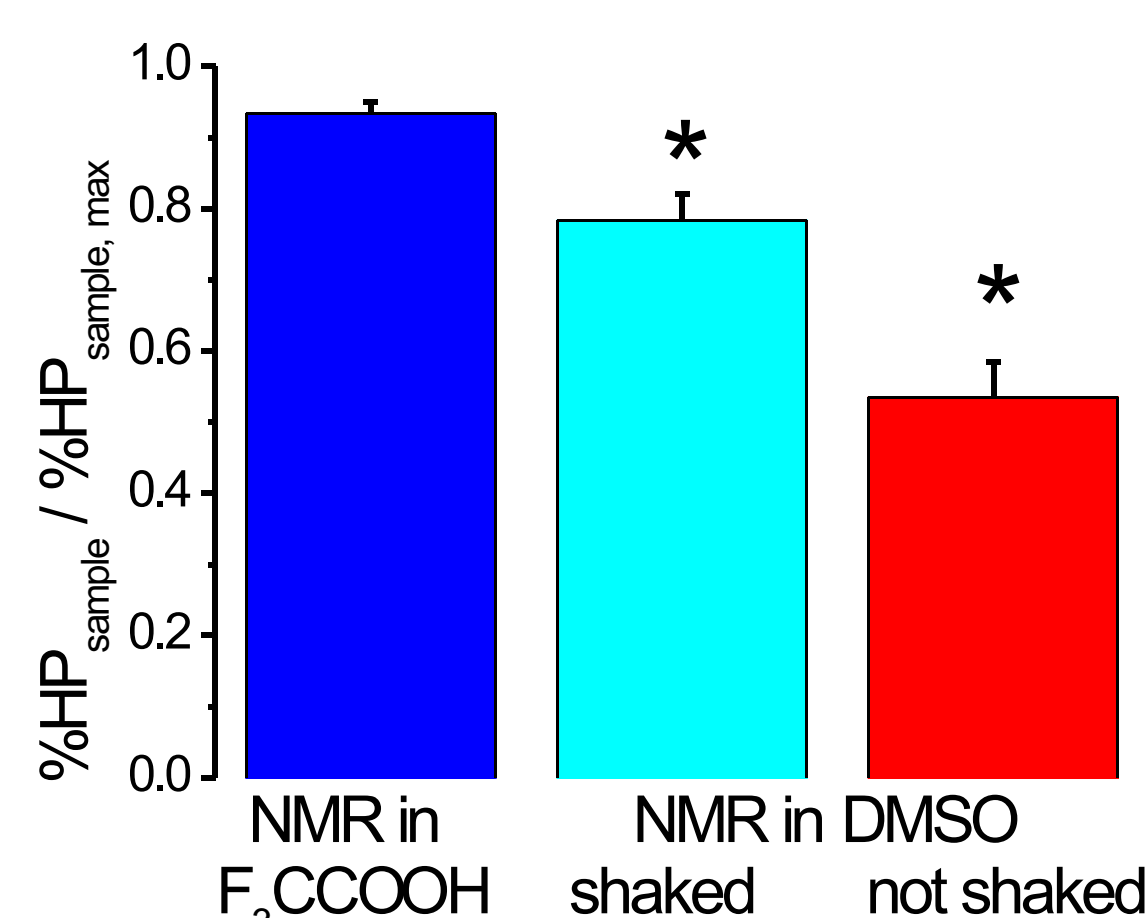


Figure 6 / 7:

This figures show two parts of the NMR-spectrum of the F₃CCOOH and the HCl method respectively. Comparing the figures, a clear difference in the relation of the signal of the sample-protons to the signal of the water-protons can be seen. Note also the slight differences in the chemical shift.

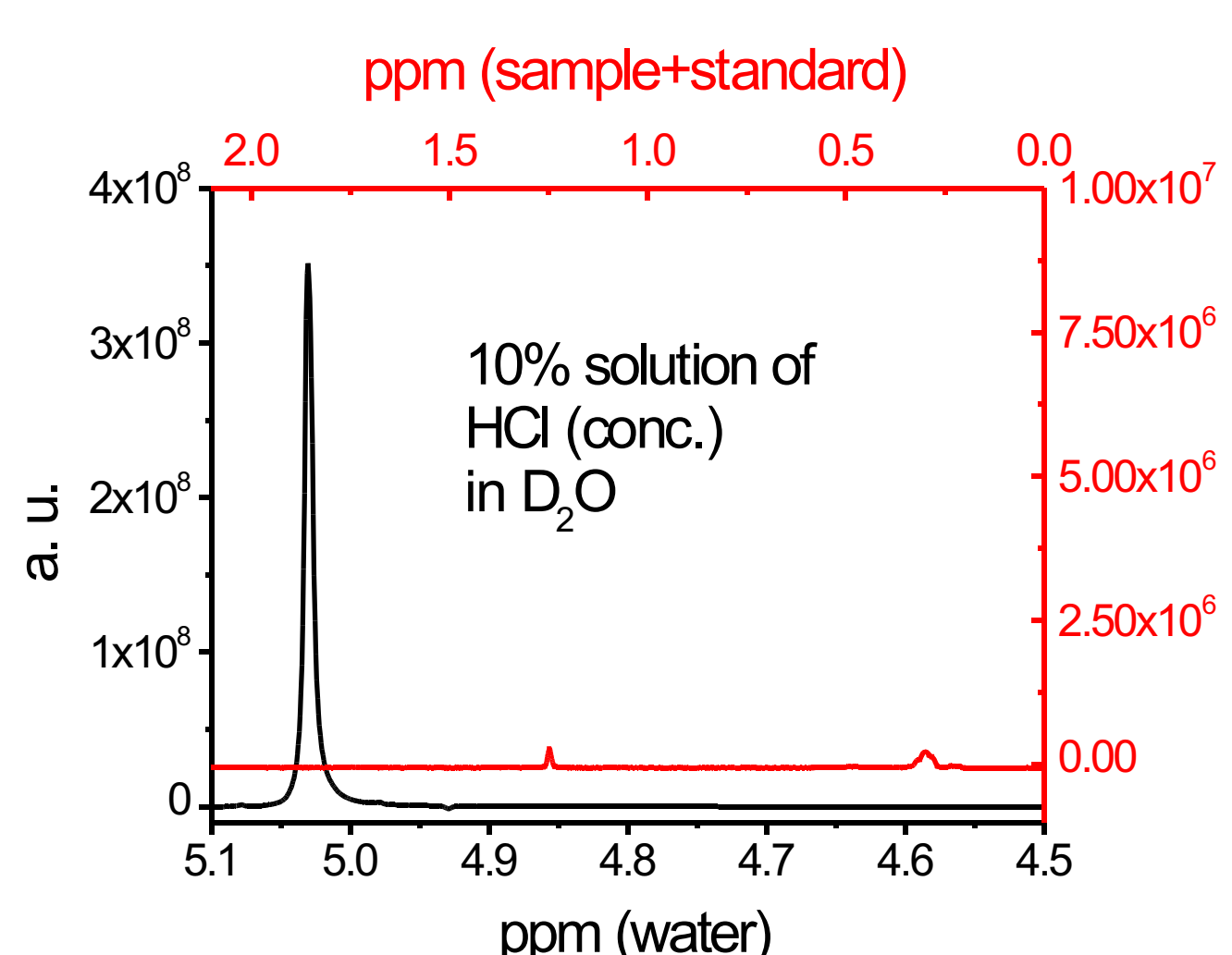
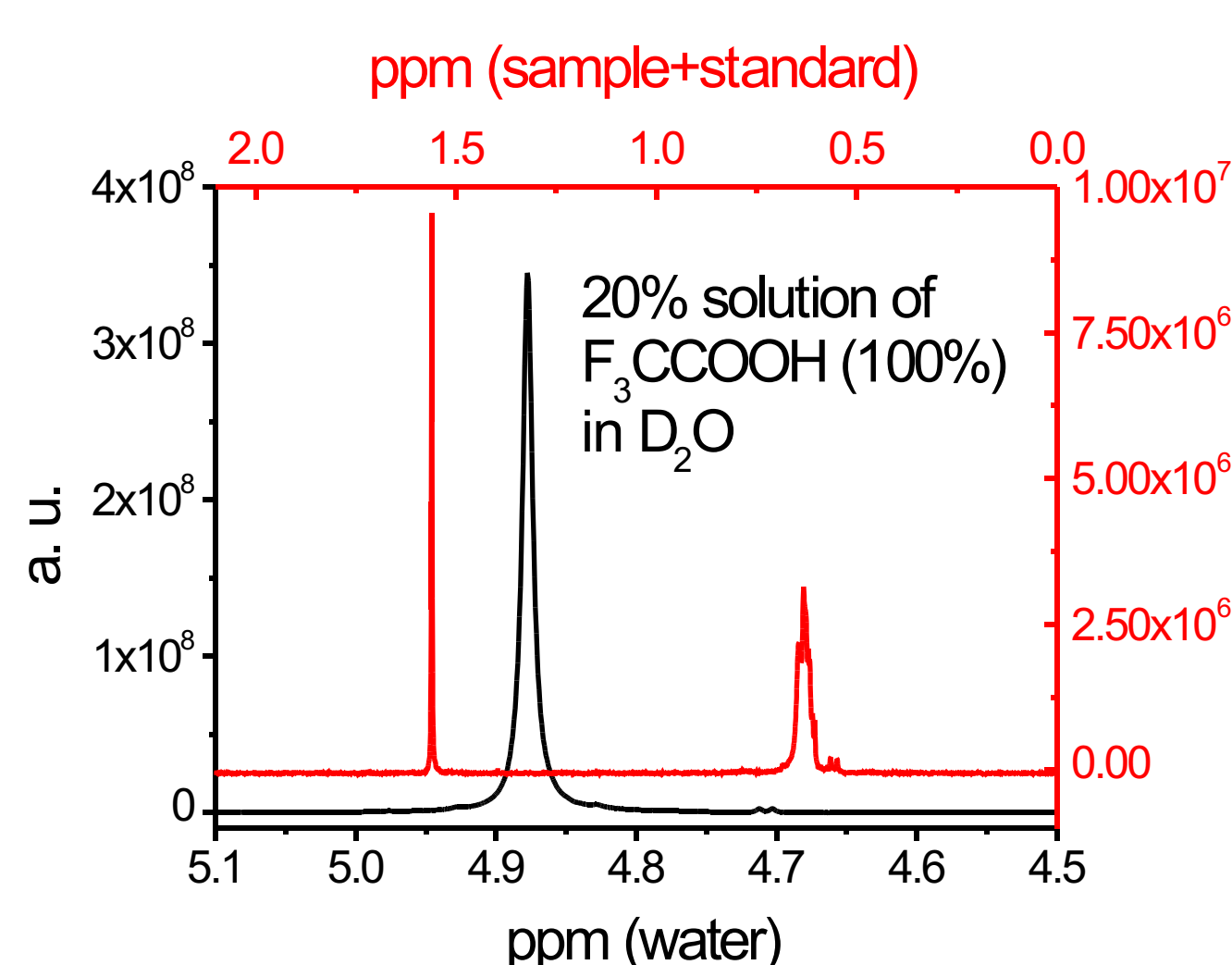


Figure 10:

The % HP values obtained with the colourimetric method were all significant lower than the values obtained with the NMR method (assumed reason: the critical 3 min-heating-period at 100 °C, References).

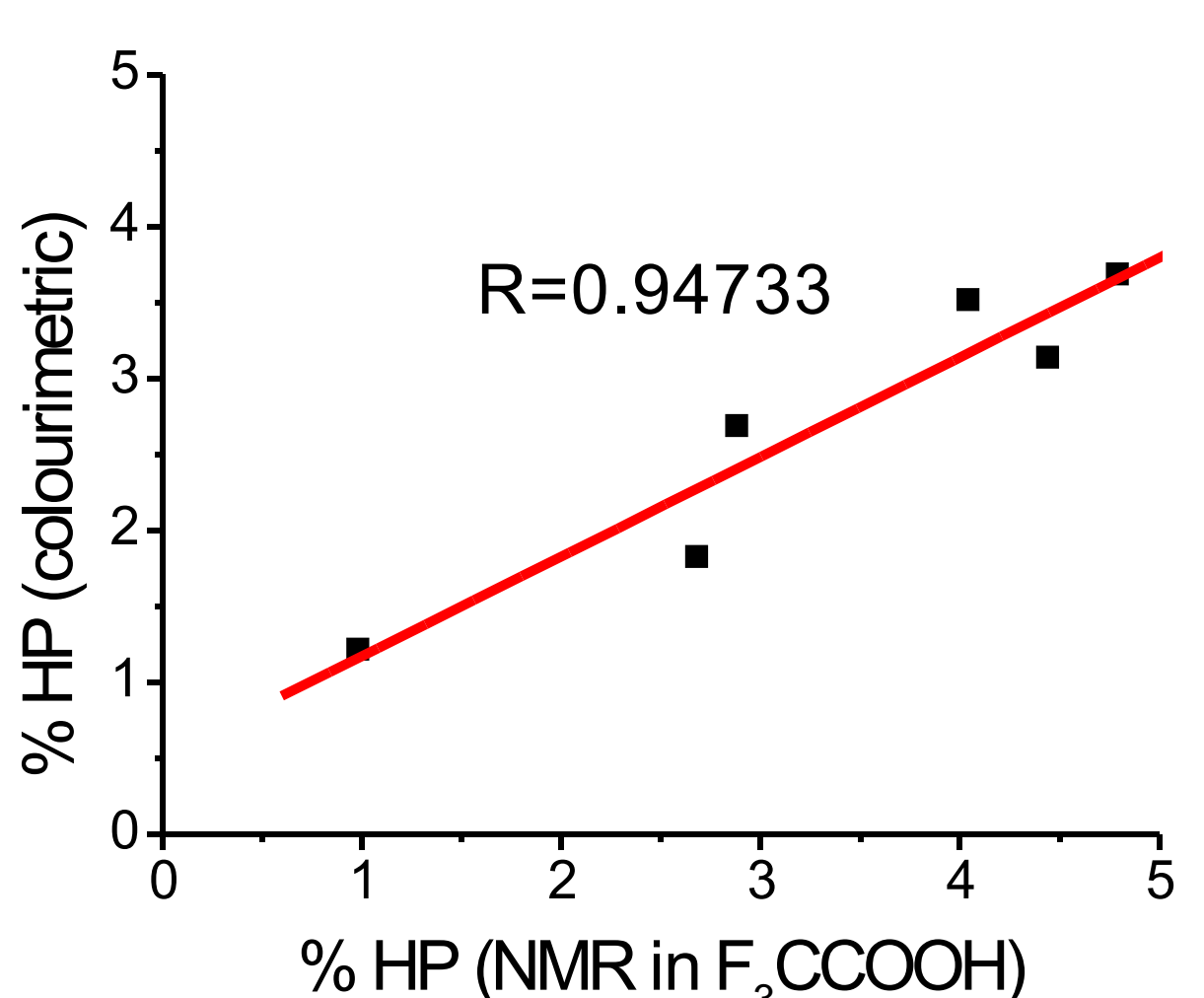
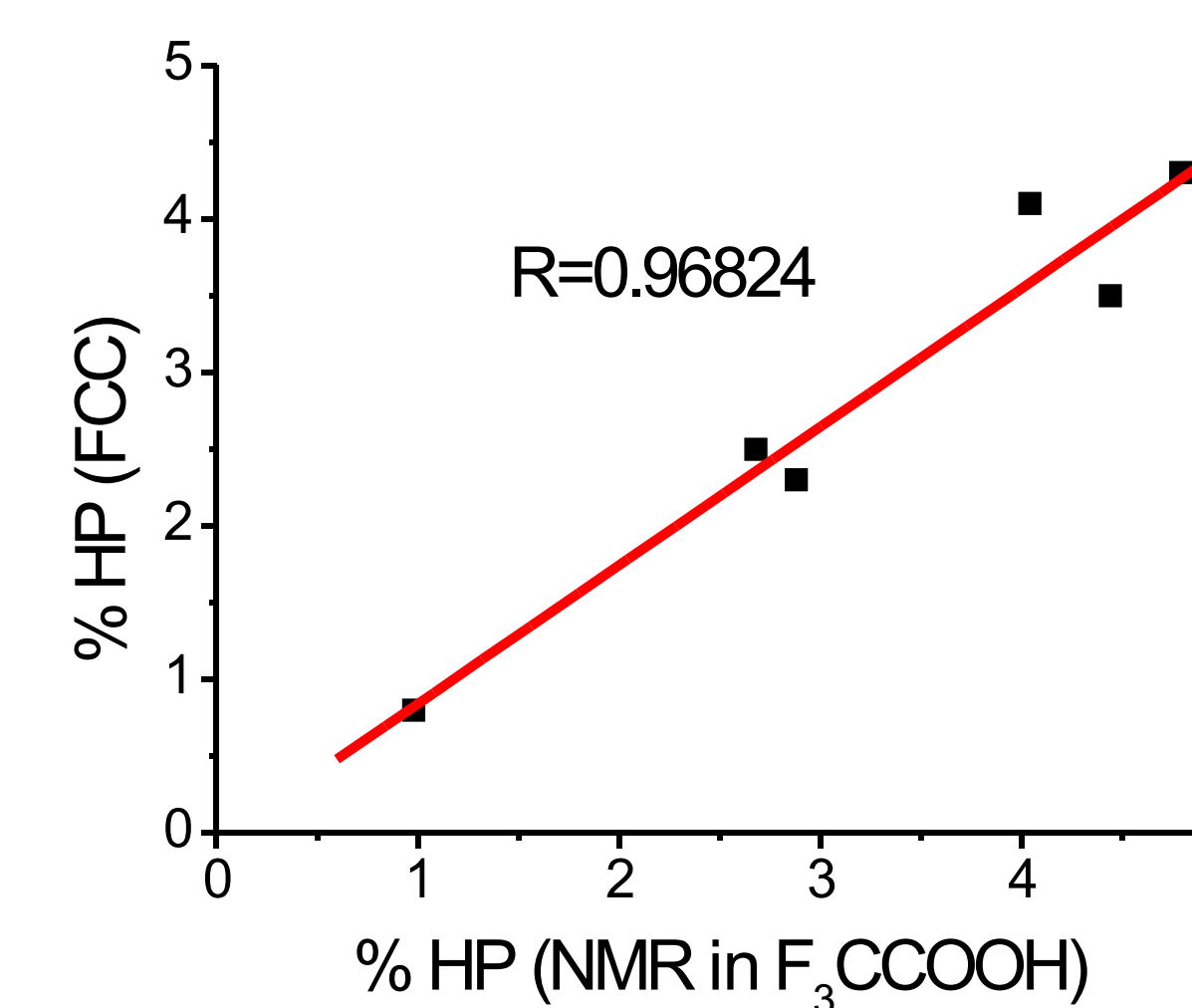


Figure 11:

The agreement between the NMR method and the FCC is worse than with other methods. In addition the dispersion (see also Table 1) of the FCC method is high.



Conclusions

For fast and approximate determination: NMR method in DMSO-d₆ (shaken) is sufficient

For accurate and fast analysis: NMR in F₃CCOOH is the recommended method, only disadvantage: expensive equipment (NMR in HCl: worse resolution because of water-signal)

IR method: alternative to NMR, fast, less experimental effort, but high dispersion and secondary standards necessary

Colourimetric method: critical heating period, high experiment time, large amount of sulfuric acid, high dispersion, but inexpensive

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- Food-Chemical-Codex III (FCC III). Pages 126-129, 514-515.

Table 1:

This table summarizes the dispersion and the experimental time of all used methods. The dispersion of the DMSO method and the FCC are both too high to get exact % HP values, the dispersion of the IR method is acceptable but also high in comparison to the other methods. The disadvantages of the colourimetric and the FCC method are their experimental times.

method	standard deviation / mean	approx. experiment time
NMR in F ₃ CCOOH	5.8 %	0.5 h
NMR in HCl	3.5 %	0.5 h
NMR in DMSO	> 15 %	0.3 h
IR	11.4 %	0.5 h
colourimetric	5.3 %	5.0 h
FCC	> 15 %	12 h