# Rapid determination of the acidity, alkalinity and carboxyl content of aqueous samples by $^1$H NMR with minimal sample quantity

Matthew Wallace,* Kevin Lam, Agne Kuraite and Yaroslav Z. Khimyak

School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.

**Corresponding Author:** matthew.wallace@uea.ac.uk

**ABSTRACT:** The titratable acidity, alkalinity and carboxylate content are fundamental properties required for the understanding of aqueous chemical systems. Here, we present a set of new methods that allow these properties to be determined directly by $^1$H NMR without the labor, cost and sample quantity associated with running separate potentiometric or conductometric titrations. Our methods require only the measurement of the pH sensitive $^1$H chemical shifts of indicator molecules and do not require the tedious titration of reagents into a sample. To determine the titratable acidity, an excess of 2-methylimidazole (2MI) is added to a sample and the quantity of protons absorbed by 2MI determined from its $^1$H chemical shifts. The titratable alkalinity of a sample can be similarly determined using acetic acid. To determine the concentration of deprotonated carboxylates, a sample is acidified with HCl and the quantity of $H^+$ absorbed determined from the $^1$H chemical shift of methylphosphonic acid. We validate our methods by demonstrating the measurement of the acidity of fruit-flavored drinks, the alkalinity of tap water and the carboxylate content of nanocellulose dispersions.

The titratable acidity and alkalinity of aqueous samples are of importance for a wide variety of fields including food science, agronomy, water treatment and engineering.[1-2] For example, the titratable acidity of fruit juice determines the flavor and can be used to judge the ripeness of crops.[1] Elsewhere, in soft matter science, the carboxylate content of colloidal systems determines their stability and pH-responsive behaviour.[3-4] The acidity, alkalinity and carboxylate content of aqueous samples are conventionally determined using potentiometric or conductometric titrations. Though simple and robust, these methods provide very limited chemical information and require large sample volumes (typically > 10 mL) which are serious drawbacks for the analysis of complex samples or custom synthesized materials.[5, 4] The detailed chemical composition of samples remains obscure while it can be impossible to detect the minor degradation of samples due to the presence of strong acid or base. In contrast, $^1$H NMR is a powerful tool to study complex mixtures such as foods or natural waters and can provide unique information on the structure and dynamics of colloidal systems.[6-8]

Here, we present a convenient set of methods that allow the acidity, alkalinity and carboxylate content of aqueous samples to be determined directly by $^1$H NMR with less than 0.6 mL of sample per measurement. We measure the acidity of apple squash (dilute apple juice with added organic acids) and standardized HCl as well as the alkalinity of tap water. Excellent concordance with potentiometric data is obtained while the chemical composition of the sample can be determined simultaneously from the same spectrum. We also demonstrate the measurement of the residual acidity and carboxylate content of functionalized cellulose nanocrystal (CNC) dispersions with less than 6 mg of solid material per measurement. The chemical stability and aggregation state of the cellulose can be simultaneously assessed by $^1$H NMR.

## EXPERIMENTAL SECTION

**Materials.** All chemicals were purchased from Sigma-Aldrich or Fisher Scientific and used as received. Milli-Q water (18.2 MΩ.cm) was used throughout the study. HCl was purchased as 0.100 M and 1.0±0.05 M standards. Apple squash concentrate was purchased from Sainsbury's Supermarkets Ltd., UK. A list of ingredients is provided in Section S-1.1. Solutions of 2-methylimidazole (2MI, 0.173±0.002 M), acetic acid (0.502±0.005 M) and disodium methylphosphonate (MPA, 40 mM) were prepared in $H_2O$ and their concentration verified by integration of the $^1$H NMR resonances against a potassium hydrogen phthalate (KHP) standard. CNC was prepared following the procedure of Yu *et al.*[9] The full synthesis is described in Section S-1.2.

**Preparation of NMR samples.** All NMR samples were prepared in 100% $H_2O$ to ensure maximum compatibility of our methods with aqueous samples. For the measurement of titratable acidity, TA (Table 2), 2MI (230 μL, 0.173 M) and 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS, 20 μL, 20 mM) were combined with apple squash (40 μL) or HCl (200 μL, 0.100 M) and the volume made up to 2000 μL with $H_2O$. Analogous reference samples were prepared without HCl or apple squash. For the measurement of titratable alkalinity, [B] (Table 3), water sample (3896 μL), acetic acid (63.7 μL, 0.502 M) and DSS (40 μL, 20 mM) were combined in a 7 mL glass vial. 550 μL of each solution was transferred to a 5 mm NMR tube for analysis. For the measurement of TA of CNC (Table 5), samples were prepared directly in 5 mm NMR tubes to conserve sample. MPA (27.5 μL, 40 mM), DSS (2.8 μL, 20 mM), DMSO (2.8 μL, 1 vol%) and CNC (3-4 wt%) were combined with $H_2O$ to give a final volume of 550 μL and a CNC concentration of 1 wt%. The samples for the measurement of the carboxylate content, COO(H), were prepared directly in 5

mm NMR tubes. For the analysis of disodium fumarate (Table 4), HCl (110 μL, 0.100 M), disodium fumarate (110 μL, 10±0.3 mM), MPA (2.8 μL, 40 mM) and DSS (2.8 μL, 20 mM) were combined and the volume made up to 550 μL with $H_2O$. After analysis, HCl (15 μL, 1.0 M) was added to the NMR tube to provide a measurement at 46 mM HCl. HCl was also added to the analogous reference sample prepared without fumarate. For the analysis of CNC (Table 5), MPA (2.8 μL, 40 mM), DMSO (2.8 μL, 1 vol%), HCl (110 μL, 0.100 M), DSS (2.8 μL, 20 mM) and CNC (3-4 wt%) were combined with $H_2O$ to give a final volume of 550 μL and a CNC concentration of 1 wt%. Analogous reference samples were prepared without CNC. To determine the quantity of citrate or malate esterified to CNC, [A], by $^1$H integration, NaOH (55 μL, 1.0 M), KHP (26.2 μL, 84.1±0.8 mM) and CNC (3-4 wt%) were combined with $H_2O$ in a 5 mm NMR tube to a volume of 550 μL and CNC concentration of 1 wt%. Samples were stood overnight at 24±1 °C before analysis.

**Potentiometric titration of apple squash and tap water.** Apple squash (500-1000 μL) or water sample (10.0±0.1 mL) was transferred to a 100 mL beaker equipped with a magnetic stirrer and 20 mL of $H_2O$ added. The sample was titrated with either 19.3±0.3 mM NaOH (apple squash) or 2.00±0.02 mM HCl (water sample) and the pH monitored using a Hanna Instruments pH210 meter equipped with an FC200 probe. The pH meter was calibrated before use with pH 4.01 and pH 7.01 buffers. The endpoint of the titrations was taken as pH 8.2 and pH 4.7 for the analysis of the apple squash and water sample respectively. For the titration of water, an acidic endpoint is necessary to determine the concentration of bicarbonate.[2] The NaOH was freshly prepared and standardized with 0.100 M HCl before use. The acidity of the apple squash concentrate was obtained by titration as 0.496±0.007 M (mean ± standard deviation, n = 3), with an overall uncertainty of 3% arising from the uncertainty in the concentration of the NaOH standard and the titre volume. Titrations of water were carried out in duplicate with concordance (±0.05 mL) obtained. The uncertainty in [B] is estimated as 3% and 6% for the tap and filtered water respectively (Section S-11).

**$^1$H NMR.** NMR experiments were performed at 298 K on a Bruker 500 MHz spectrometer. The temperature was calibrated using a methanol standard and can be assumed accurate to 0.5 K,[10] the variation in temperature with time being less than 0.1 K. All spectra were recorded off-lock in 100% $H_2O$ using the perfect echo WATERGATE sequence of Adams *et al.*[11] incorporating the double echo W5 sequence of Liu *et al.*[12] The delay between successive pulses in the selective pulse train was set at 333 μs. The 90° pulse was set at 12 μs. The signal acquisition time and relaxation delay were 4.37 s (64K data points) and 1.0 s respectively. 8 dummy scans and 16 scans were acquired for the measurement of TA (Table 2) and [B] (Table 3), and 32 scans for the measurement of COO(H) (Tables 4 and 5). A relaxation delay of 40 s was used for the determination of [A] (Table 5). An uncertainty in [A] of 5% is assumed based on the analysis of a 4 mM sodium citrate standard. All spectra are referenced to DSS (0 ppm) and processed with 128K points and an exponential line broadening factor of 1 Hz. A line broadening factor of 3 Hz was used for the calculation of COO(H) and [$H_3O^+$] due to the low concentration of MPA. The $^1$H imaging experiment to extract the indicator parameters of 2MI is described in Section S-2.

## RESULTS AND DISCUSSION

**Determination of titratable acidity by $^1$H NMR.** Our methods require only the measurement of the $^1$H NMR chemical shifts of indicator molecules.[13-14] Fast exchange on the NMR timescale causes the observed chemical shift, $\delta_{obs}$, to be a population weighted average of the limiting protonated ($\delta_H$) and deprotonated ($\delta_L$) forms of the indicator. $\delta_{obs}$ is related to the pH of the solution *via* Equation 1:[13-14]

$$\mathrm{pH} = \mathrm{pK_a} + \log_{10}\left(\frac{\delta_{obs} - \delta_H}{\delta_L - \delta_{obs}}\right) \qquad 1$$

where the p$K_a$ is that of the indicator. We note that Equation 1 has been widely used to measure the pH of aqueous samples and can provide compartment-specific pH values in complex systems such as living cells.[15-16] The concentration of protonated indicator, [HInd], is related to the total indicator concentration, $[\mathrm{Ind}]_{total}$, by Equation 2:

$$[\mathrm{HInd}] = [\mathrm{Ind}]_{total}\left(\frac{\delta_{obs} - \delta_L}{\delta_H - \delta_L}\right) \qquad 2$$

The titratable acidity, TA, is defined as the total concentration of acidic protons in a sample.[5, 1] In our method, a solution of a basic indicator is added to a sample so that all of the acidic protons are quantitatively absorbed by the indicator. TA is therefore approximately equal to [HInd]. However, to correct for any $CO_2$ absorption by the indicator stock solution, it is necessary to measure the chemical shift in a reference sample comprising the indicator alone, $\delta_{ref}$. TA is thus provided by Equation 3:

$$\mathrm{TA} = [\mathrm{Ind}]_{total}\left(\frac{\delta_{obs} - \delta_{ref}}{\delta_H - \delta_L}\right) + \left(\frac{\delta_H - \delta_{ref}}{\delta_{ref} - \delta_L}\right)10^{\mathrm{pK_a} - \mathrm{pK_w}} \qquad 3$$

The right-hand term corrects for the deprotonation of $H_2O$ by the indicator in the reference sample. p$K_w$ is taken as 13.95 with no ionic strength correction necessary (Section S-4). 2-methylimidazole (2MI) is used as the indicator for the measurement of TA. The low basicity of 2MI is sufficient to deprotonate carboxylic acids (p$K_a$ < 6), although we note that less acidic species can contribute to the apparent titratable acidity (Section S-10).The $^1$H chemical shifts were measured as a function of pH using chemical shift imaging (Fig. 1, a) as described in our previous work and in Section S-2.[13] Excellent fits to Equation 1 are obtained, confirming the validity of the fast exchange model and allowing the p$K_a$ and limiting chemical shifts of 2MI to be determined.

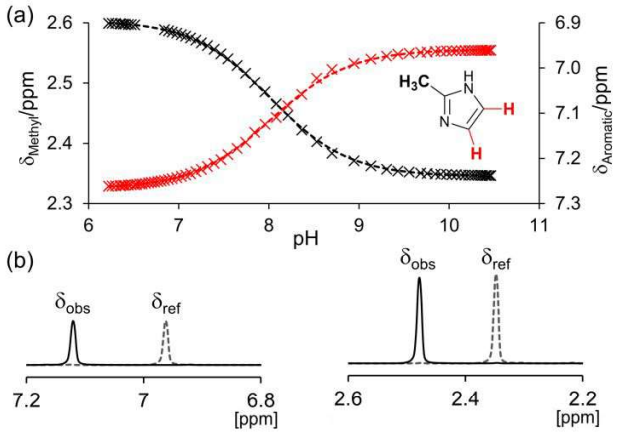



**Fig. 1.** (a) Plot of chemical shift of methyl (black) and aromatic (red) resonance of 2MI versus pH. Fits to Equation 1 are shown as dashed lines. The right-hand axis has been reversed for clarity. (b) Partial $^1$H NMR spectra of aromatic (left) and methyl (right) resonances of 2MI in 10 mM HCl ($\delta_{obs}$, solid line) and reference sample ($\delta_{ref}$, dashed). $[2MI]_{total}$ = 20 mM in both spectra.

The methyl and aromatic chemical shifts of 2MI are very sensitive to protonation and no broadening of the resonances is detected when the 2MI is partially protonated with HCl (Fig. 1, b). The two resonances can therefore be used together to provide extra confidence in the acidity measurements obtained from Equation 3. We note that fast exchange between the tautomeric forms of the imidazole leads to equivalence of the aromatic protons. The indicator parameters of 2MI are provided in Table 1. We note that disodium methylphosphonate (MPA) and acetic acid can be used to measure the pH of aqueous samples from pH 1 to pH 9 and are used in this work to quantify the titratable alkalinity and carboxylate content.[13] For the samples and ionic strengths discussed in this work, the pH determined from Equation 1 using the parameters listed in Table 1 can be assumed accurate to 0.1 units when the pH is 9 or below, and to 0.3 units at pH 10 (Section S-3).[13] We note that binding to proteins or other macromolecules is likely to cause a broadening of the $^1$H resonances of the indicators.[17-18] The interaction and interference of these sample components with the indicators can therefore be detected by analysis of $^1$H linewidths (Fig. S-8 and S-11).

**Table 1. p$K_a$ values and limiting chemical shifts of indicators used in this work**

| Indicator | p$K_a$ (ionic strength/mM) | $\delta_H$/ppm | $\delta_L$/ppm |
|---|---|---|---|
| 2MI[a] | 8.02 (25) | 2.6044 (methyl)<br>7.2673 (aromatic) | 2.3450<br>6.9586 |
| MPA[b,c] | 7.75 (41)<br>2.32 (20) | 1.2819<br>1.5106 | 1.0711<br>1.2819 |
| Acetic acid[b] | 4.72[d] (10) | 2.0830 | 1.9060 |

[a]Determined as described in Section S-2. [b]Parameters taken from Reference 13. [c]The protonation steps of MPA are treated separately due to the large difference in p$K_a$ (Fig. S-2). [d]p$K_a$ value calculated at 10 mM ionic strength (Section S-6). All parameters determined in 100% $H_2O$ at 25 °C. p$K_a$ determined at ionic strength indicated.

To test Equation 3, samples were prepared containing 0.2 mM DSS, 20±0.4 mM 2MI and either 0 or 10 mM standardised HCl. Another sample was prepared containing 20 µL/mL of apple squash in place of the HCl. The squash contained 20% apple juice with added citric and malic acids. TA measured by $^1$H NMR is provided in Table 2.

**Table 2. Acidities of apple squash and standardised HCl determined by $^1$H NMR[a]**

| Sample | $\delta_{obs}$/ppm | pH[b] | TA/mM[c] |
|---|---|---|---|
| Reference | 2.3487 (methyl)<br>6.9632 (aromatic) | 9.86<br>9.84 | 0.20±0.21[d]<br>0.22±0.18[d] |
| HCl<br>10.0±0.1 mM | 2.4790<br>7.1207 | 7.99<br>7.98 | 10.13±0.31<br>10.28±0.29 |
| Apple squash<br>20 µL/mL | 2.4806<br>7.1225 | 7.98<br>7.97 | 10.25±0.31<br>10.40±0.29<br>(9.91±0.30)[e] |

[a]Values are calculated using the methyl (upper) and aromatic (lower) resonances of 2MI (20 mM). [b]Calculated using Equation 1. [c]Uncertainties estimated *via* a propagation of error analysis (Section S-4). [d]Calculated using Equation S-6. [e]Determined by potentiometric titration with NaOH.

The concentration of HCl determined by NMR is within 3% of the known concentration which is in agreement with a propagation of error analysis (Section S-4). The acidity of apple squash is within 5% of the value determined by potentiometric titration. Similar agreement is reported between conventional potentiometric titrations and alternative methods for the determination of TA, such as flow-based analyses[5, 19] or miniaturized potentiometric titrations.[1] The TA and chemical composition of the apple squash can be determined from the same $^1$H NMR spectrum, thus conserving time and sample (Fig. S-8). The apparent acidity measured in the reference sample corresponds to a $CO_2$ contamination of 1 mol% which has negligible impact upon the accuracy of the method (Fig. S-4). The close agreement between the pH and TA determined using the methyl and the aromatic resonances of 2MI confirms that there is no significant interaction between 2MI and components of the apple squash. The interaction of 2MI with other species would affect the methyl and aromatic resonances to different extents (Fig. S-8).[20] The endpoints of conventional potentiometric titrations are typically between pH 7 and pH 9.[1, 5] In our method, the pH will lie within this range and acidity measurements will be obtained within 10% uncertainty provided the ratio $TA/[Ind]_{total}$ is between 0.1 and 0.9 (Fig. S-3 and S-5). At 20 mM 2MI, TA may thus be measured over the range 2 - 18 mM. The limiting chemical shifts of NMR pH indicators in $H_2O$ typically vary by less than 0.002 ppm when the ionic strength is below 0.1 M and only monovalent ions are present.[13-14] The measurable range of acidity can therefore be adjusted by varying the concentration of indicator used and the method validated with HCl (Fig. S-3 and S-5).

**Determination of titratable alkalinity.** An excess of an acidic indicator such as acetic acid is added to an alkaline sample.

Assuming the base in the sample reacts quantitatively with the indicator, the titratable alkalinity, [B], is given by Equation 4:

$$[B] = [Ind]_{total}\left(\frac{\delta_H - \delta_{obs}}{\delta_H - \delta_L}\right) - \left(\frac{\delta_{obs} - \delta_L}{\delta_H - \delta_{obs}}\right)10^{-pK_a} \quad 4$$

The term on the right corrects for the self-dissociation of the acidic indicator (Section S-6). To test our method, samples were prepared containing 8.0±0.2 mM acetic acid, 0.2 mM DSS and 974 μL/mL of tap water collected from a supply in Norwich, UK. Another sample was prepared where the water had been passed through a domestic Brita® MAXTRA cartridge. Alkalinities of the water samples determined by NMR and by potentiometric titration with HCl are compared in Table 3.

**Table 3. Alkalinities of water samples**

| Sample | $\delta_{obs}$/ppm | pH[a] | [B]/mM[b] (NMR) | [B]/mM (Potentiometric) |
|---|---|---|---|---|
| Tap water | 1.9797 | 4.87 | 4.78±0.12[c] | 4.82±0.14 |
| Brita® filter | 2.0635 | 3.81 | 0.75±0.11[c] | 0.59±0.04 |
| Milli-Q | 2.0727 | 3.51 | 0.16±0.18 | - |

[a]pH of sample with 8.0 mM acetic acid, calculated using Equation 1. [b]Uncertainties estimated *via* a propagation of error analysis (Section S-6). [c]Values scaled by 1/0.974 for comparison with potentiometric data.

The alkalinities of the tap water sample measured by NMR and by potentiometric titration correspond to 239±6 and 241±7 mg/L $CaCO_3$ respectively, in good agreement with the 234 mg/L $CaCO_3$ reported by the local water authority (Section S-12). $HCO_3^-$ is the dominant alkaline species in our tap water sample.[2] The p$K_a$ of $CO_2$ can be taken as 6.29 at an ionic strength of 10 mM and $HCO_3^-$ will therefore be >95% protonated below pH 5.[21] When acetic acid is used as an indicator, the pH will lie below 5 if the ratio $[B]/[Ind]_{total}$ is below 0.7 while acceptable experimental uncertainty (< 6 %) will be achieved if $[B]/[Ind]_{total} > 0.2$ (Fig. S-6). With 8 mM acetic acid, [B] may thus be measured over the range 1.6 to 5.6 mM. More acidic indicators give greater uncertainties due to the right-hand term of Equation 4 (Fig. S-6). For Brita® filtered water, the discrepancy in the alkalinity determined using the two techniques is attributable to the experimental uncertainty when $[B]/[Ind]_{total} < 0.2$ (Fig. S-6) and the errors associated with performing potentiometric titrations at low ionic strength and alkalinity (Section S-11). The apparent alkalinity of Milli-Q water is within the uncertainty of the measurement. We note that our method is not significantly affected by the low levels of $Ca^{2+}$ or $Mg^{2+}$ present in natural waters due to the low affinity of acetate for these ions (Section S-6).[22, 14]

**Determination of the deprotonated carboxylate content.** A sample is acidified with HCl and dibasic MPA added as an indicator. A reference sample comprising only HCl and MPA is also prepared. The concentration of free $H^+$ ions, $[H_3O^+]$, in sample and reference is determined from the $^1$H chemical shift of MPA ($\delta_{obs}$ and $\delta_{ref}$, respectively). Provided a low concentration of MPA is used so that [HCl] >> [MPA], the quantity of $H^+$ absorbed by the sample is equal to the difference in $[H_3O^+]$ between sample and reference. The carboxylate content can be assumed equal to the quantity of $H^+$ absorbed by the sample, provided the pH of the sample following acidification is sufficiently low to ensure full protonation. If the sample contains no ions in addition to the carboxylates and monovalent counterions, the ionic strength, I, and the dissociation constant of MPA, $K_{eff}$, in sample and reference will be equal. $K_{eff}$ is calculated from $\delta_{ref}$ using Equation 5:

$$K_{eff} = \left([HCl] - [MPA]_{total}\left[1 + \frac{\delta_{ref} - \delta_L}{\delta_H - \delta_L}\right]\right)\left(\frac{\delta_H - \delta_{ref}}{\delta_{ref} - \delta_L}\right) \quad 5$$

where [HCl] is the total concentration of HCl added to the sample and reference. The pH is determined from Equation 1 using the lower p$K_a$ value and limiting chemical shifts of MPA (Table 1). The carboxylate content, COO(H), is calculated using Equation 6:

$$COO(H) = K_{eff}\left(\frac{\delta_{ref} - \delta_L}{\delta_H - \delta_{ref}} - \frac{\delta_{obs} - \delta_L}{\delta_H - \delta_{obs}}\right) \quad 6$$

A derivation of Equations 5 and 6 is provided in Section S-7. Samples were prepared containing 2.0 mM disodium fumarate, 0.2 mM MPA, 0.1 mM DSS and 20 mM HCl. After analysis, [HCl] was increased to 46 mM by the addition of 15 μL of 1.0 M HCl to the NMR tube. Reference samples were also prepared. The carboxylate contents of the samples are provided in Table 4.

**Table 4. Carboxylate content of 2.0 mM fumarate**

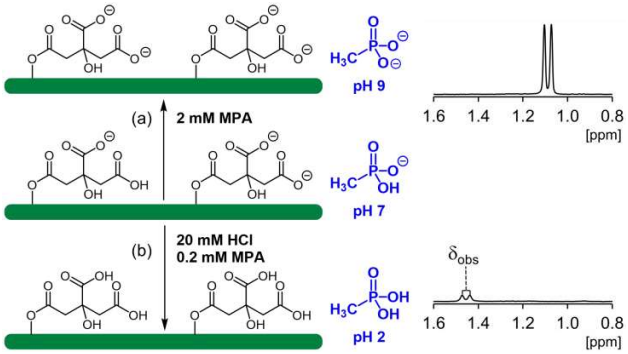
| [HCl] /mM | pH[a] | COO(H)/mM (predicted)[b] | COO(H)/mM (NMR) | $[H_3O^+]$ /mM[c] |
|---|---|---|---|---|
| 20±0.2 | 1.84 | 3.84 | 3.87±0.30 | 15.8±0.3 |
| 46±1.4 | 1.47 | 3.82 | 3.85±1.2 | 41.8±1.8 |

[a]pH of fumarate sample calculated using Equation 1. [b]Quantity of $H^+$ absorbed by fumarate, calculated using the CurTiPot package.[23] [c]$[H_3O^+]$ in fumarate sample determined by NMR (Equation S-22): $[H_3O^+]_{sample} = [H_3O^+]_{ref}$ - COO(H). Uncertainties in $[H_3O^+]$ and COO(H) estimated by propagation of error analysis (Section S-7).

The measured carboxylate contents agree closely with the theoretical values. Fumaric acid possesses p$K_a$ values of 2.99 and 4.42 (I = 0).[13] 2 mM fumarate is therefore 96% protonated in 20 mM HCl and > 98% protonated in 46 mM HCl. However, the increased experimental uncertainty at 46 mM HCl more than outweighs any benefit arising from the more complete protonation of the sample and [HCl] of 20 mM is recommended (Fig. S-7). Nevertheless, a second measurement at higher [HCl] can be used to validate the measurement of COO(H) at 20 mM HCl as the difference in $[H_3O^+]$ between the samples is equal to the difference in [HCl] (Table 4). $K_{eff}$, $\delta_H$ and $\delta_L$ are therefore equal in the sample and reference, confirming the validity of Equation 6. The absence of a significant interaction between MPA and other sample components can be further verified by analysis of $^1$H linewidths and $^{31}$P-$^1$H couplings (Fig. S-11). If the pH of the sample following acidification with 20 mM HCl is > 2, additional HCl can be added to attain a more complete protonation of the sample and thus a more accurate determination of the carboxylate content (Fig. S-7). Finally, we note that our method is expected to be tolerant of low concentrations of $Ca^{2+}$ or $Mg^{2+}$ due to the low affinity of

these ions for monobasic MPA, in analogy with dihydrogen phosphate.[22] However, the sample and reference used for the determination of COO(H) must have the same ionic strength for Equation 6 to be valid.

**Measurement of the carboxylate content and residual titratable acidity of nanomaterials.** Functionalization of cellulose nanocrystals (CNC) with carboxylic acids *via* esterification is an attractive route to prepare stable aqueous dispersions that are pH and ion responsive.[24, 9] However, conventional conductometric titrations for the determination of the carboxylate content require large quantities of sample per measurement (typically 0.1 g) that may not be available.[4, 3] Hydrolysis of the cellulose esters may also occur during the titrations which could interfere with the measurements obtained and be impossible to detect. In contrast, our methods require less than 6 mg of CNC per measurement while any low molecular weight hydrolysis products can be readily detected by $^1$H NMR. To test our methods, 1 wt% aqueous dispersions of CNC functionalized with citric or malic acid were prepared at pH 7.4. The residual acidity was determined by the addition of 2 mM dibasic MPA (Equation 3) using the upper limiting chemical shifts of MPA provided in Table 1. The concentration of deprotonated carboxylates at pH 7.4 was determined *via* acidification with 20 mM HCl (Equations 5 and 6) using 0.2 mM MPA as an indicator (Scheme 1).




**Scheme 1.** Strategy to determine the residual acidity (a) and deprotonated carboxylate content (b) of neutral CNC dispersions by $^1$H NMR using dibasic MPA as an indicator.

The total quantity of citrate or malate esterified to CNC was determined by alkaline hydrolysis in 0.1 M NaOH. 4.0±0.1 mM potassium hydrogen phthalate was included to allow the quantity of citrate or malate released, [A], to be determined by integration of their $^1$H resonances. Data is provided in Table 5.

**Table 5. Analysis of 1 wt% CNC dispersions**

| Sample | TA/mM[a] | pH[b] | COO(H) /mM[a] | pH[c] | [A]/mM[d] |
|---|---|---|---|---|---|
| Citrate CNC | 0.15±0.02 | 8.79 | 3.47±0.30 | 1.83 | 1.62±0.08 |
| Malate CNC | 0.09±0.02 | 9.02 | 2.68±0.31 | 1.81 | 2.78±0.14 |

[a]Uncertainties estimated *via* a propagation of error analysis (Section S-4 and S-7). [b]pH of 1 wt% CNC in 2 mM MPA, calculated using Equation 1. [c]pH of 1 wt% CNC in 20 mM HCl, calculated using Equation 1. [d]Concentration of free citrate or malate measured by $^1$H NMR after alkaline hydrolysis of CNC.

The neutral CNC dispersions have negligible titratable acidity indicating that the carboxylate groups are essentially fully (> 95%) deprotonated at pH 7.4. We note that dibasic MPA has a significant affinity for divalent cations such as $Ca^{2+}$ or $Mg^{2+}$ and is not recommended for the determination of TA when these ions are present.[25] Comparing the concentration of deprotonated carboxylates at pH 7.4, COO(H), with the concentration of free citrate or malate measured after alkaline hydrolysis, it is apparent that the carboxylic acids are esterified to the cellulose *via* one carboxylate group. CNC functionalized with citric acid thus bears two carboxylate groups per citrate unit, in agreement with solid-state NMR data presented by Spinella *et al*.[24] No free citrate or malate could be detected in the samples used for the determination of TA and COO(H) indicating negligible hydrolysis of the CNC during the experiments (Fig. S-9 and S-10). We note that DMSO can be used as an alternative $^1$H chemical shift reference (Section S-1.3). The uncertainty in the determination of COO(H) by NMR is comparable to the uncertainty of conventional conductometric titrations[3, 9, 24] or acid-base titrimetry.[26]

## CONCLUSIONS

Our methods allow the titratable acidity, alkalinity and carboxylate content of samples to be determined with an accuracy comparable to other published methods but with all the additional chemical information afforded by a standard $^1$H NMR spectrum. The methods avoid the need for tedious manual titrations while typically consuming less than 10% of the sample quantity. We anticipate our methods will find particular use in the analysis of colloidal systems where only small quantities of sample are available and chemical understanding is paramount. Our methods are suitable for the high-throughput analysis of samples by $^1$H NMR using robotic sample changers. It is likely that the methods could be adapted to work on low field benchtop NMR instruments (Section S-4, S-6 and S-7).

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website:

Further experimental details; use of DMSO as chemical shift reference; analysis of 2MI by imaging; propagation of uncertainty analysis of Equations 1-6; effect of $CO_2$ and acid identity on TA; $^1$H NMR spectra of apple squash, HCl, tap water and CNC; effect of acid p$K_a$ on TA measurements; simulated titration curves of water samples; water quality report for supply used in this study (PDF)

Research data will be available at: https://people.uea.ac.uk/en/datasets/data-for-Rapid-determination-of-the-acidity-alkalinity-andcarboxyl-content-of-aqueous-samples-by-1H-NMR-with-minimal sample-quantity

## AUTHOR INFORMATION

### ORCID

Matthew Wallace: 0000-0002-5751-1827

Yaroslav Khimyak: 0000-0003-0424-4128

**Author Contributions**

All authors have given approval to the final version of the manuscript.

## ACKNOWLEDGMENTS

MW thanks the Royal Commission for the Exhibition of 1851 for a Research Fellowship. MW also thanks the Royal Society for a Research Grant: RGS\R1\191336. We are grateful for the use of the University of East Anglia (UEA) Faculty of Science NMR facility.

## REFERENCES

1. Sonsa-ard, T.; Nacapricha, D.; Kaneta, T., Miniaturized Potentiometric Titration for Improving Portability and Accuracy in the Determination of Total Acid in Squeezed Fruit Juice. *J. Food Sci.* **2019,** *84*, 2165-2170.
2. Neal, C., Alkalinity measurements within natural waters: Towards a standardised approach. *Sci. Total Environ.* **2001,** *265*, 99-113.
3. Kawaguchi, S.; Yekta, A.; Winnik, M. A., Surface characterization and dissociation properties of carboxylic acid core-shell latex particle by potentiometric and conductometric titration. *J. Colloid Interface Sci.* **1995,** *176*, 362-369.
4. Foster, E. J.; Moon, R. J.; Agarwal, U. P.; Bortner, M. J.; Bras, J.; Camarero-Espinosa, S.; Chan, K. J.; Clift, M. J. D.; Cranston, E. D.; Eichhorn, S. J.; Fox, D. M.; Hamad, W. Y.; Heux, L.; Jean, B.; Korey, M.; Nieh, W.; Ong, K. J.; Reid, M. S.; Renneckar, S.; Roberts, R.; Shatkin, J. A.; Simonsen, J.; Stinson-Bagby, K.; Wanasekara, N.; Youngblood, J., Current characterization methods for cellulose nanomaterials. *Chem. Soc. Rev.* **2018,** *47*, 2609-2679.
5. Vahl, K.; Kahlert, H.; Von Mühlen, L.; Albrecht, A.; Meyer, G.; Behnert, J., Determination of the titratable acidity and the pH of wine based on potentiometric flow injection analysis. *Talanta* **2013,** *111*, 134-139.
6. Liu, S.; Armes, S. P., Synthesis and aqueous solution behavior of a pH-responsive schizophrenic diblock copolymer. *Langmuir* **2003,** *19*, 4432-4438.
7. Hatzakis, E., Nuclear Magnetic Resonance (NMR) Spectroscopy in Food Science: A Comprehensive Review. *Compr. Rev. Food Sci. F.* **2019,** *18*, 189-220.
8. Whitty, S. D.; Waggoner, D. C.; Cory, R. M.; Kaplan, L. A.; Hatcher, P. G., Direct noninvasive 1H NMR analysis of stream water DOM: Insights into the effects of lyophilization compared with whole water. *Magn. Reson. Chem.* **2019**, 1-14. https://doi.org/10.1002/mrc.4935
9. Yu, H. Y.; Zhang, D. Z.; Lu, F. F.; Yao, J., New Approach for Single-Step Extraction of Carboxylated Cellulose Nanocrystals for Their Use As Adsorbents and Flocculants. *ACS Sustain. Chem. Eng.* **2016,** *4*, 2632-2643.
10. Ammann, C.; Meier, P.; Merbach, A., A simple multinuclear NMR thermometer. *J. Magn. Reson.* **1982,** *46*, 319-321.
11. Adams, R. W.; Holroyd, C. M.; Aguilar, J. A.; Nilsson, M.; Morris, G. A., "Perfecting" WATERGATE: Clean proton NMR spectra from aqueous solution. *ChemComm* **2013,** *49*, 358-360.
12. Liu, M.; Mao, X. A.; Ye, C.; Huang, H.; Nicholson, J. K.; Lindon, J. C., Improved Watergate Pulse Sequences for Solvent Suppression in NMR Spectroscopy. *J. Magn. Reson.* **1998,** *132*, 125-129.
13. Wallace, M.; Adams, D. J.; Iggo, J. A., Titrations without the Additions: The Efficient Determination of pKa Values Using NMR Imaging Techniques. *Anal. Chem.* **2018,** *90*, 4160-4166.
14. Tynkkynen, T.; Tiainen, M.; Soininen, P.; Laatikainen, R., From proton nuclear magnetic resonance spectra to pH. Assessment of 1H NMR pH indicator compound set for deuterium oxide solutions. *Anal. Chim. Acta.* **2009,** *648*, 105-112.
15. Moon, R. B.; Richards, J. H., Determination of intracellular pH by 31P magnetic resonance. *J. Biol. Chem.* **1973,** *248*, 7276-7278.
16. Cohen, J. S.; Motiei, M.; Carmi, S.; Shiperto, D.; Yefet, O.; Ringel, I., Determination of Intracellular pH and Compartmentation Using Diffusion-Weighted NMR Spectroscopy with pH-Sensitive Indicators. *Magn. Reson. Med.* **2004,** *51*, 900-903.
17. Shortridge, M. D.; Hage, D. S.; Harbison, G. S.; Powers, R., Estimating protein-ligand binding affinity using high-throughput screening by NMR. *J. Comb. Chem.* **2008,** *10*, 948-958.
18. Wallace, M.; Iggo, J. A.; Adams, D. J., Probing the surface chemistry of self-assembled peptide hydrogels using solution-state NMR spectroscopy. *Soft Matter* **2017,** *13*, 1716-1727.
19. Jakmunee, J.; Rujiralai, T.; Grudpan, K., Sequential injection titration with spectrophotometric detection for the assay of acidity in fruit juices. *Anal. Sci.* **2006,** *22*, 157-160.
20. Yu, X. Y.; Cai, S. H.; Xu, X.; Chen, Z., NMR and theoretical study on the coordination and solution structures of the interaction between diperoxovanadate complexes and histidine-like ligands. *Inorg. Chem.* **2005,** *44*, 6755-6762.
21. Park, S. N.; Kim, C. S.; Kim, M. H.; Lee, I. J.; Kim, K., Spectrophotometric measurement of the first dissociation constants of carbonic acid at elevated temperatures. *J. Chem. Soc. Faraday Trans.* **1998,** *94*, 1421-1425.
22. Daniele, P. G.; Foti, C.; Gianguzza, A.; Prenesti, E.; Sammartano, S., Weak alkali and alkaline earth metal complexes of low molecular weight ligands in aqueous solution. *Coord. Chem. Rev.* **2008,** *252*, 1093-1107.
23. Gutz, I. G. R., CurTiPot – pH and Acid–Base Titration Curves: Analysis and Simulation freeware, version 4.2, http://www.iq.usp.br/gutz/Curtipot_.html.
24. Spinella, S.; Maiorana, A.; Qian, Q.; Dawson, N. J.; Hepworth, V.; McCallum, S. A.; Ganesh, M.; Singer, K. D.; Gross, R. A., Concurrent Cellulose Hydrolysis and Esterification to Prepare a Surface-Modified Cellulose Nanocrystal Decorated with Carboxylic Acid Moieties. *ACS Sustain. Chem. Eng.* **2016,** *4*, 1538-1550.
25. DeFronzo, M.; Gillies, R. J., Characterization of methylphosphonate as a 31P NMR pH indicator. *J. Biol. Chem.* **1987,** *262*, 11032-11037.
26. Wen, C. Y.; Sun, J. Y., Quantitative Determination of the Carboxyl Groups on Individual Nanoparticles by Acid-Base Titrimetry. *ChemistrySelect* **2017,** *2*, 10885-10888.

For Table of Contents only