Application of Good's buffers to pH imaging using hyperpolarized 13C-MRI

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Target audience: Physicians and scientists engaged in hyperpolarized ^{13}C MRI research, and any with interest in interstitial pH imaging.

Purpose: Solid tumors have numerous mechanisms to export acid into the extracellular space, resulting in a low interstitial pH. This feature of the tumor microenvironment has been interrogated by several MRI based methods, including ^{1}H, ^{31}P, ^{19}F, and hyperpolarized ^{13}C MR spectroscopy, with typical reported tumor pH values ranging from 6.5 – 7.0 (1). However, these methods suffer from various limitations including low spatial resolution, low signal to noise, and long scan times. In a landmark 1966 paper, Good set out several properties which would produce an ideal biochemical pH buffer, including near-neutral pKa, high solubility, impermeability to membranes, biochemical inertness, and ease of preparation, and prepared several buffers fulfilling these criteria (2). We rationalized that a molecule with these properties would yield an ideal interstitial pH imaging probe. In this abstract, we describe preliminary application of ^{13}C,^{15}N ACES, one of Good's buffers, to pH imaging using hyperpolarized ^{13}C MRI. This method combines the large ^{13}C chemical shifts of HP ACES in the physiologic range, with the dramatic signal enhancements afforded by dissolution dynamic nuclear polarization (DNP).

Methods: Several Good's buffers were analyzed by ^{13}C NMR spectroscopy on a Varian Unity Inova 500 MHz system with 250 mM solutions at pH 7.4 and 6.5 with urea as a chemical shift standard. Based on these results, ^{13}C,^{15}N-ACES was obtained from Isotec. Optimal glass-forming preparations used 0.95 equivalents of a 10N sodium hydroxide solution, or one equivalent of sodium glycinate in water, yielding concentrations of 3.7M and 2.2M, respectively. Solid-state polarization was optimized with respect to Gd-DOTA concentration, polarization time, and microwave frequency, and was performed on a HyperSense polarizer (Oxford instruments). A copolarization method was developed based on previously described technique, using ^{13}C,^{15}N urea as a chemical shift standard (3). Percent polarization and T_1 values were calculated as previously described. Hyperpolarized ^{13}C,^{15}N ACES solution was diluted by a factor of 10 at different pH values, in a five-compartment phantom, and the chemical shifts were analyzed by 2D-CSI on a 3T GE Signa MRI. Finally, 2 mL of the hyperpolarized ^{13}C,^{15}N ACES solution was intravenously administered to a wild type rat and imaged on a 3T MRI using 3D EPSI, with 1 cm^3 voxels.

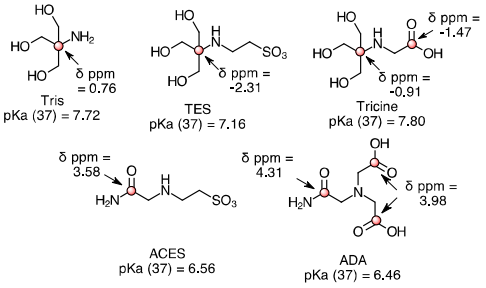

Figure 1. Good's buffers analyzed by thermal equilibrium ^{13}C NMR. Polarizable nuclei are marked in red, and the chemical shift difference between the compounds at pH 7.4

Results: The difference between the chemical shifts of candidate long-T_1 carbons of several Good's buffers at pH 7.4 and 6.5 is summarized in **Figure 1**. We found that physiologic calcium concentration abrogated the pH dependent chemical shift of ADA (data not shown) and thus ^{13}C,^{15}N-ACES was selected as a pH probe (**Fig 2A**). A pH:chemical shift titration curve obtained using urea as a standard demonstrated a remarkable pH dependent chemical shift change over the physiologic range (**Fig 2B**). Using this titration curve, our obtained pKa of 6.60 is in agreement with the previously published value of 6.56 at 37 degrees (2). Using optimized polarization parameters, we obtained average polarization of 12.5%. Calculated T_1 was 18s at 11.7 T, and 25s at 3T. A copolarization method using ^{13}C,^{15}N ACES and ^{13}C,^{15}N urea as a chemical shift standard was applied to imaging in model systems. Due to the large pH dependent chemical shift, we were easily able to detect subtle pH differences, both in a 5mm NMR tube at 11.7T and in a 3T phantom imaging experiment (**Fig 2C - E**). Next, we applied ACES to imaging in a wild type rat, with the probe well-tolerated at 50 mM. Using this method, we were able to identify both ACES and urea signal in the abdomen (**Fig 2F**).

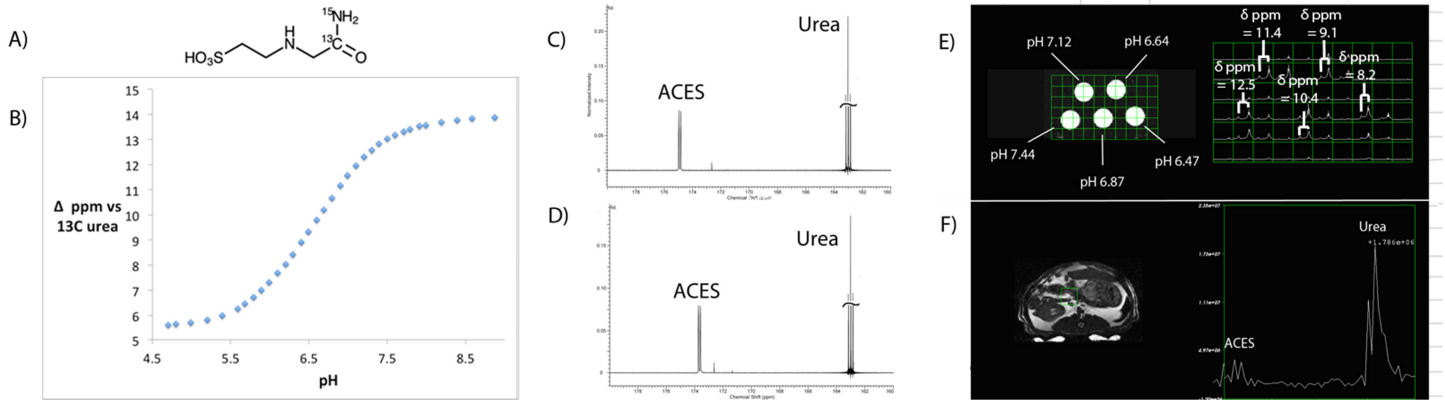

Figure 2. A) Structure of ^{13}C,^{15}N ACES. **B)** Plot of chemical shift of ACES minus the chemical of urea versus pH, at 37 degrees. **C & D)** Hyperpolarized ^{13}C spectrum obtained on a 500 MHz NMR following copolarization of ^{13}C, ^{15}N urea and ^{13}C, ^{15}N ACES. A peak corresponding to hyperpolarized natural abundance glycine (used in sample preparation) is also seen. Calculated pH based on chemical shift was 7.16 (C) and 6.86 (D), in agreement with the values of 7.22 and 6.91 measured in a pH meter. **E)** Five compartment phantom imaged at 3T, demonstrating ability to discriminate pH under imaging conditions. **F)** 3D chemical shift imaging of a rat following injection. A representative spectrum over the mid abdomen is depicted containing both hyperpolarized ^{13}C, ^{15}N urea and ^{13}C, ^{15}N ACES signal.

Discussion and conclusions: Of the Good's buffers studied, ACES proved to be the optimal choice for pH imaging due to large pH dependent chemical shift and biochemical inertness (**Fig 1A, 2B**). Using optimized polarization parameters, we were able to detect subtle pH changes over the physiologic range (**Fig 2 C-E**). Importantly, we were able to detect hyperpolarized ^{13}C, ^{15}N ACES signal *in vivo* (**Fig 2F**), potentially enabling its use as an interstitial pH imaging probe in murine models of malignancy. Because HP ACES is a biological buffer, this probe also has excellent potential for clinical translation.

References: 1. Hashim AI, Zhang X, Wojtkowiak JW, et al. Imaging pH and metastasis. NMR Biomed. 2011; 24:582-91.
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