Comments on “Thermodynamics of *cis,cis*-muconic acid solubility in various polar solvents at low temperature range”

Jack M. Carraher,^{a,b} John E. Matthiesen,^{a,b} Jean-Philippe Tessonier^{a,b,*}

^a Department of Chemical and Biological Engineering, Iowa State University, Ames, Iowa, United States

^b NSF Engineering Research Center for Biorenewable Chemicals (CBiRC), Ames, Iowa, United States

*Corresponding author. Email: tesso@iastate.edu Phone: +1 515-294-4595

ABSTRACT

New light is shed on results published by Scelfo, Pirone, and Russo in “Thermodynamics of *cis,cis*-muconic acid solubility in various polar solvents at low temperature range”. We show that under the conditions described in their article, *cis,cis*-muconic acid isomerizes to *cis,trans*-muconic acid within minutes. This isomerization can be easily overlooked as both isomers present similar retention times during liquid chromatography analysis. Yet, the consequences are significant as the *cis,trans* isomer is five times more soluble at room temperature under acidic conditions than *cis,cis*-muconic acid. pH should also be measured and taken into account as this parameter dramatically influences the species in solution, hence the solubility limit.

KEYWORDS: *cis,cis*-Muconic acid; *cis,trans*-Muconic acid; Isomerization; Solubility; Aqueous solution; pH

1. INTRODUCTION

Muconic acid (MA) has recently emerged as a promising biobased intermediate for the sustainable production of adipic acid, terephthalic acid, and other monomers central to the production of various commodity polyamides and polyesters such as Nylon 6,6 and polyethylene terephthalate (PET), an estimated market value of 118 billion U.S. dollars per year.[1, 2] MA is currently gathering significant interests from both industry [3-5] and academic research groups worldwide [6-8]. Substantial progress has already been achieved to produce MA from plant-based biomass by fermentation using metabolically engineered yeasts and bacteria. The designed microbes were shown to convert cellulosic sugars and lignin monomers to *cis,cis*-muconic acid (ccMA) with high yield and titer. Further conversion of the ccMA intermediate to the desired renewable diacid monomer is performed through chemical reactions, typically in the presence of a heterogeneous catalyst [8-17].

Advancements in downstream chemical conversion of MA to adipic, terephthalic, and other diacids are also conditioned by the development of a fundamental understanding of the MA

chemistry and an accurate determination of its physicochemical properties. It is therefore crucial that data published on this key intermediate is based on appropriately validated experimental and analytical techniques. We address here several errors related to the experimental and analytical methods that have been published by Scelfo *et al.* which, unfortunately, significantly impact their solubility results [18].

2. RESULTS AND DISCUSSION

MA is a six carbon atom diunsaturated dicarboxylic acid structurally similar to adipic acid. However, muconic acid exists in three different isomers depending on the configuration of its carbon-carbon double bonds: *cis,cis*-, *cis,trans*-, and *trans,trans*-muconic acid (respectively ccMA, ctMA, and ttMA). These isomers have vastly different solubility at low pH at which they are fully protonated (ccMA ~1g/L, ctMA = 5.2 g/L, and ttMA = 90 mg/L) [14]. The three isomers are easily distinguished by ^1H nuclear magnetic resonance (NMR) and representative ^1H NMR spectra of a ccMA and ctMA mixture are shown in Figure 1a. However, their separation and quantification using high pressure liquid chromatography (HPLC) is delicate. We believe that the HPLC method and column selected by Scelfo *et al.* could be insufficient for separating ccMA from ctMA and could yield flawed solubility data. Our HPLC analysis using an Aminex HPX-87H column (BioRad) at 30 °C with a 0.30 mL/min flow rate of 5 mM H_2SO_4 indicates that the isomers cannot be separated with this setup (Figure 1b). The Aminex HPX-87H column is very similar to the column used by the authors (REZEX ROH+ organic acid) and we suspect that it is equally unsuitable for cc- and ctMA separation, particularly at 70 °C with a flow rate of 0.6 mL/min described in their article. It is, therefore, understandable that the authors assumed that they were observing only ccMA.

Figure 1. a) ^1H NMR spectra of ccMA (orange) conversion to ctMA (green) at 22.5 °C as a function of time. b) HPLC chromatograms of genuine ccMA and ctMA acquired on a Waters e2695 HPLC equipped with a photodiode array detector. The conditions were the following: Aminex HPX-87H column (BioRad), 0.30 mL/min 5 mM H_2SO_4 , column temperature 30 °C. c) Kinetic trace of ccMA conversion to ctMA at 83 °C (pH ~3.5).

With the three isomers in mind, one must consider transformations and potential equilibria between them and how conditions will affect not only the thermodynamics but also the kinetics.

The specific kinetic data has not yet been published. However, the literature reports that isomerization of the cc- isomer to the ct- isomer occurs readily (in as little as 10 minutes under reflux) [10, 19]. We reported a half-life for this isomerization of less than 1 day at room temperature [14]. This means that within the 4.5 hours that ccMA was ‘allowed to equilibrate’ in Scelfo *et al.*’s study, nearly 13% would have been converted to ctMA. Our current work, which has not yet been submitted for publication, shows that even at temperatures as low as 30 °C the half-life of ccMA⁻ (singly deprotonated ccMA) is only 137 minutes and 816 minutes for ccMA. The isomerization is significantly faster at 75 °C ($t_{1/2}$ ~ 1 minute and 17 minutes for ccMA⁻ and ccMA, respectively). A kinetic trace of ccMA conversion (83 °C at pH ~3.5) is shown in Figure 1c. At this pH, a mixture of ccMA, ccMA⁻ and ccMA²⁻ (fully deprotonated ccMA) is present. ccMA²⁻ is stable at all temperatures. We do not fault the authors for not knowing the specific rate constants as they have not been made public. However, the authors cite several works in which the ready isomerization to ctMA is mentioned and yet they did not take this transformation into consideration during experimental design.

Finally, the solubility of any acid most certainly is dependent upon the pH of the solution; the protonation state has a significant effect on the solubility due to differences in the molecule’s charge and interactions with the solvent. For instance, we have observed that ctMA exhibits a solubility of 5.2 g/L at pH < 2 while ctMA²⁻ (pH >6.5) presents a solubility of more than 100 g/L [20]. Likewise, at low pH the solubility of ccMA was found to be ~ 1 g/L while Drath’s was able to obtain titers as high as 59.2 g/L ccMA²⁻ at elevated pH [16].

All of the above arguments hold true in other solvents with the exception of pH effects. Our ¹H NMR work indicates that fully protonated MA is the most prevalent form in other solvents.

We value the efforts of the authors to contribute important fundamental data on MA. It is clear that MA is a much more complicated molecule than its analog adipic acid and, therefore, these studies require more intense scrutiny and additional considerations relative to those of adipic acid. To aid in future product analysis, we have detailed a procedure to quantify the three isomers of MA with baseline separation as shown in Fig S1a within the reference [14]. This method is explicitly detailed in Table 1.

Table 1. Liquid chromatography method to separate MA isomers

Instrument	Water Acquity H-Class UPLC
Column	Waters HSS C18 (1.8 µm, 2.1 X 100 mm) 0.2 % Formic Acid/Water (95%), Methanol
Isocratic Mobile Phase	(5%)
Flow Rate	0.45 ml min ⁻¹
Column Temperature	45 °C
Injection Volume	8 µl
Detector	Waters Acquity PDA Detector set at 230 nm

As a general strategy for separation, in particular for reverse phase chromatography, an acidic buffer is required for MA retention and subsequent separation to occur. As a rule of thumb, the higher the organic content of the mobile phase the faster the elution time. While the separation of isomers presents some difficulty, the molar absorptivity for this compound is large making UV detection very sensitive. RI is also suited for this application, but with less sensitivity.

It should also be noted that this is one outlined procedure and we anticipate that the three MA isomers could also be separated with ion exclusion and normal phase chromatography under the right conditions. Due to the increasing popularity of this molecule, as the authors point out, each method could likely have its own benefits and disadvantages in the near future.

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