Pyruvic Acid Polymerization at Physiological pH and Characterization of Its Degradation Products
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Purpose
To study the polymerization of pyruvic acid at different temperatures and concentrations at around physiological pH. The development of a HPLC method to resolve pyruvic acid from its degradation products and their identification by NMR and LC/MS techniques.

Methods
A series of stability studies of 20 mM and 50 mM pyruvic acid solutions were conducted over six months at 25°C, 40°C, 50°C and 70°C, in 25 mM phosphate buffer at pH 7.4 and ionic strength 0.15 M. Pyruvic acid was resolved from its degradations products by HPLC on a Phenomenex Synergi 4μm Hydro-RP 80Å 250 x 4.6 mm column thermostated at 30°C. An isocratic separation was performed using 25 mM NaH2PO4 with pH 3 as mobile phase at a flow rate of 0.7 mL/min. The UV detection of pyruvic acid and its degradation products was carried out at 220 nm. To further study and characterize the degradation products a 3M pyruvic acid solution was prepared and kept in water bath at 25°C for two weeks. The major fractions were collected and analyzed by LC/MS.

Results
The percentage of intact pyruvic acid after six months at different temperatures and concentrations are shown in table 1. The proton, carbon and 2D NMR (figure 1) of the 3M reaction after two weeks have shown the presence of the dimer as the major product resulting from an aldol reaction (scheme 1). The fraction with a retention time of 6.2 minutes, which was suspected to be the dimerization product of pyruvic acid, was collected and analyzed by LC/MS. The major ion was m/z 174.8 (negative ion mode), which corresponds to the [M-1] for the dimer of pyruvic acid.

Conclusion
Pyruvic acid is not stable in concentrated solutions. The aldol-like condensation of pyruvic acid at pH 7.4 was observed to be faster at elevated temperatures and concentrations, as expected for a bimolecular reaction. HPLC, NMR and LC/MS analysis confirmed the structure of the dimer and the presence of oligomers of pyruvic acid. Further studies are needed to investigate the dimerization and polymerization reactions.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>3M</th>
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<th>20M</th>
<th>40M</th>
</tr>
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<tbody>
<tr>
<td>25°C</td>
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<td>50°C</td>
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<tr>
<td>70°C</td>
<td>40%</td>
<td>60%</td>
<td>70%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Table 1: Stability studies of 20 mM and 50 mM pyruvic acid solutions conducted for six months at 25°C, 40°C, 50°C and 70°C in 25 mM phosphate buffer at pH 7.4. The results are represented as % intact pyruvic acid.