Stopped Flow Analysis

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History

- First described by Chance (1940) and Gibson & Milnes (1964), with a view to probing enzyme kinetics
- Oscilloscopes were the first detectors

Overview

- **Stopped flow** is a type of flow-injection analysis
- Reactants are rapidly mixed in a mixing chamber, and the flow is stopped with the reactant stream in the flow cell (detector – usually some form of photometry)
- Allows
  - better measurement sensitivity due to increased residence time
  - reduced fluid dispersion by stopping flow

- **Dead time** – the time between the end of mixing and the start of measurement Typically ms range
  - Measured electronically
  - Shorter is better
Scope

- Small sample volumes (< 0.7 to 500 μL)
- Fast analysis (3 to 60 s)
- \( t_{1/2} \) of 1-10 seconds possible
- Sensitivity depends on detector
- Able to tolerate corrosive compounds (e.g. Br₂, H₂SO₄)
- Many instruments commercially available

Other developments

- Wide temperature ranges (e.g. +100 to -100 °C)
- High pressure (2 kbar)
  - measurements of activation volume
- Stopped flow with fast scan spectroscopy or with a temperature jump
  - determine transient reaction intermediates

Applications

- Enzymes
- Protein folding
- Redox reactions
- Coordination chemistry
- Catalysis (e.g. polymerization reactions)
Typical Instrument

Schematics

Drive ratio can be conveniently varied (Harvey).

Detectors

- Usually photometric detectors
  - UV/Vis & IR
  - Fluorescence (more sensitive)
  - Chemiluminescence
  - Circular dichroism (good for protein work)
  - Refractive index
  - NMR* (e.g. $^1$H, $^{19}$F)
  - EPR
  - Voltammetry
  - Electrical conductivity
Stopped Flow NMR

- NMR is the most information-rich type of spectroscopy
- Hand-mixing of reagents – useful to get data points within tens of seconds
- Stopped flow is useful to get data points within 2-10 s
- Main delay in this technique is the spin-lattice relaxation time, $T_1$
  - Reduced by addition of a relaxation agent, e.g. $\text{Cr(acac)}_3$, but causes line broadening
- Apparatus not commercially available yet

Green, D. B.; Lane, J.; Wing, R. Appl. Spectrosc. 1987, 41, 847.
Probing Ligand Substitutions

- 200 ms dead time
- No relaxation agent needed
- Calculated values agree with literature:
  \[ k_2 = 1.2 \times 10^{-2} \text{M}^{-1} \text{s}^{-1} \]
  \[ t_1 = 60 \text{ s} \]

Green, D. B.; Lane, J.; Wing, R. Appl. Spectrosc. 1987, 41, 847.

Studying the Mechanism of Catalytic Polymerization

- Landis et al. studied the Zr-catalyzed polymerization of 1-hexene
- Commercial NMR flow probe modified to give mixing chamber & related stopped flow apparatus
- Three reagent concentrations varied: 1-hexene, and 2 catalyst precursors \((rac-(C_2H_4(1\text{-indenyl})_2)ZrMe_2 \text{ & } B(C_6F_5)_3)\)
- Multiple “shots” used to sample the course of each reaction for every set of parameters

Data

Resting catalyst

Zr pre-catalyst

Active catalyst

### Analysis

<table>
<thead>
<tr>
<th>entry</th>
<th>Initiation $k_i/M^{-1}s^{-1}$</th>
<th>Propagation $k_p/M^{-1}s^{-1}$</th>
<th>Chain Transfer $k_t/s^{-1}$</th>
<th>Re-initiation $k_r/M^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.224 (2)</td>
<td>13.2 (1)</td>
<td>0.0317 (4)</td>
<td>2.70 (7)</td>
</tr>
<tr>
<td>2a</td>
<td>0.218 (2)</td>
<td>18.6 (2)</td>
<td>0.0345 (4)</td>
<td>4.12 (6)</td>
</tr>
<tr>
<td>3a</td>
<td>0.255 (1)</td>
<td>18.3 (1)</td>
<td>0.0303 (3)</td>
<td>2.56 (3)</td>
</tr>
<tr>
<td>4a</td>
<td>0.222 (3)</td>
<td>17.8 (2)</td>
<td>0.0264 (5)</td>
<td>1.62 (5)</td>
</tr>
<tr>
<td>5a</td>
<td>0.314 (3)</td>
<td>21.8 (2)</td>
<td>0.0369 (6)</td>
<td>2.33 (7)</td>
</tr>
<tr>
<td>6a</td>
<td>0.215 (2)</td>
<td>15.2 (1)</td>
<td>0.0339 (4)</td>
<td>1.80 (3)</td>
</tr>
<tr>
<td>global fit (COPASI)</td>
<td>0.2353 (6)</td>
<td>16.83 (6)</td>
<td>0.0323 (2)</td>
<td>2.06 (2)</td>
</tr>
<tr>
<td>Rapid Quenched Flow (25 °C)</td>
<td>0.25</td>
<td>8.1</td>
<td>0.0132</td>
<td>$&gt;10k_p$</td>
</tr>
<tr>
<td>Rapid Quenched Flow (0 °C)</td>
<td>0.033</td>
<td>2.2</td>
<td>0.00066</td>
<td>$&gt;10k_p$</td>
</tr>
<tr>
<td>Kinetic Modelling</td>
<td>0.031</td>
<td>3.7</td>
<td>0.0024</td>
<td>$10^3k_p$</td>
</tr>
</tbody>
</table>

Catalytic Pathway

- Re-initiation of hydridoborurate (6), originally thought to be fast, found to be very slow
- 43% of catalyst (3) inactive

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Questions