Raman Spectroscopy for Process Monitoring of Biocatalytic Synthesis of a Nonsteroidal Anti-Inflammatory Drug

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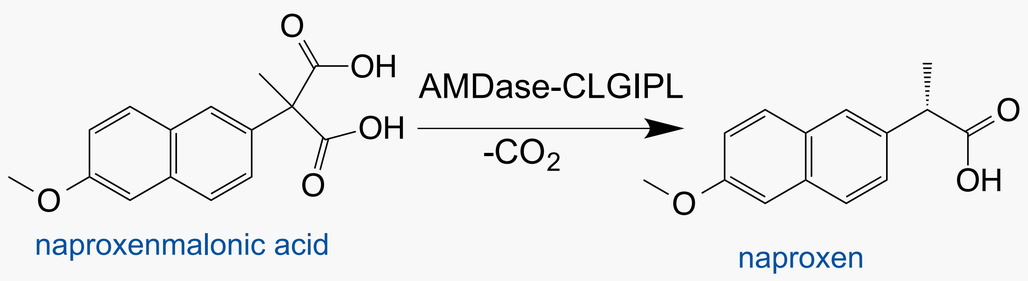
Reaction of Interest

- Naproxen: nonsteroidal anti-inflammatory drug
- Biocatalyzed naproxen synthesis: sustainable process with mild conditions
- Biocatalyst: arylmalonate decarboxylase (AMDase), mutant CLGIPL selective to pharmaceutically active *(S)*-enantiomer[1]



Reaction scheme of biocatalytic naproxen synthesis

Process Raman Spectroscopy

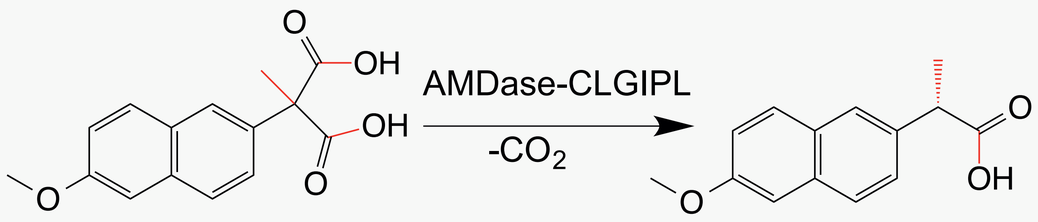
- Reactor: lightproof stirred tank reactor
- Spectrometer: MultiSpec® Raman with fiber-coupled probe (tec5 AG), immersed in reaction mixture

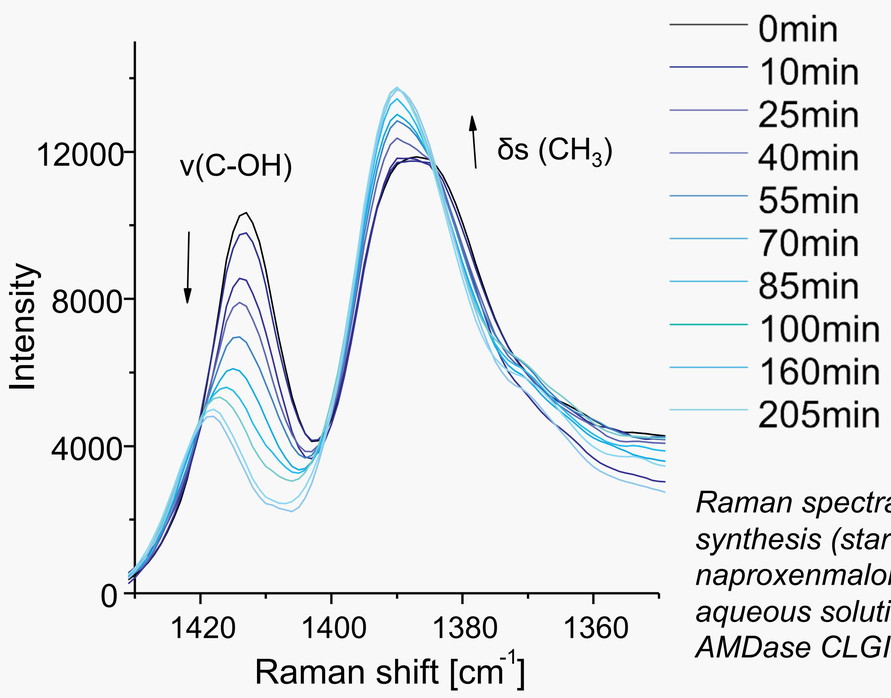
MultiSpec® Raman Spectrometer

- Parameters:
  - λ_{exc}: 785 nm, P: 500 mW,
  - $t_{integration}$: 30 s
- Pretreatment:
  - cutting (370 - 1450 cm^{-1}),
  - baseline correction concave rubberband (64 points, 10 iterations)

Raman Spectra

- Spectral range chosen for analysis: 1430 – 1350 cm^{-1}
- Two intense Raman bands interpreted as
  - symmetric deformation vibration δs (CH_3)
  - valence vibration of carboxylic acid C-OH ν(C-OH)





Raman spectra during naproxen synthesis (starting material naproxenmalonic acid 25 mM in aqueous solution, pH 8, 30 °C, AMDase CLGIPL 80 mg/l)

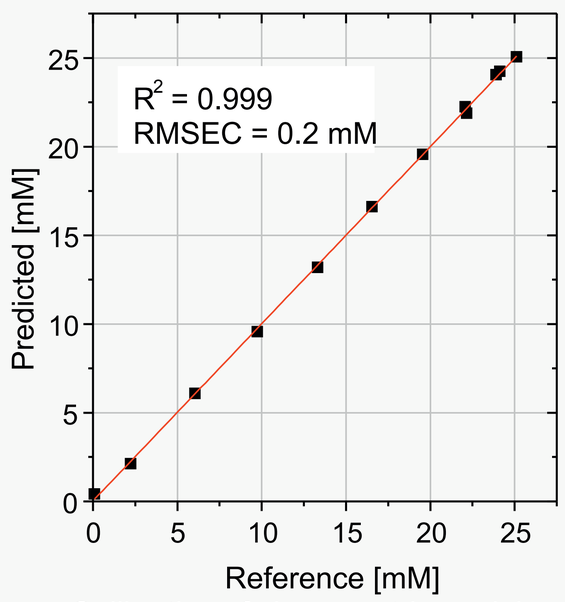
Chemometrics

- Oflline samples analyzed with HPLC
- Calibration of chemometric model with Partial Least Squares (PLS) algorithm
- Validation samples not included in PLS model
- Root mean square errors (RMSE, C: calibration, P: prediction) for model evaluation:

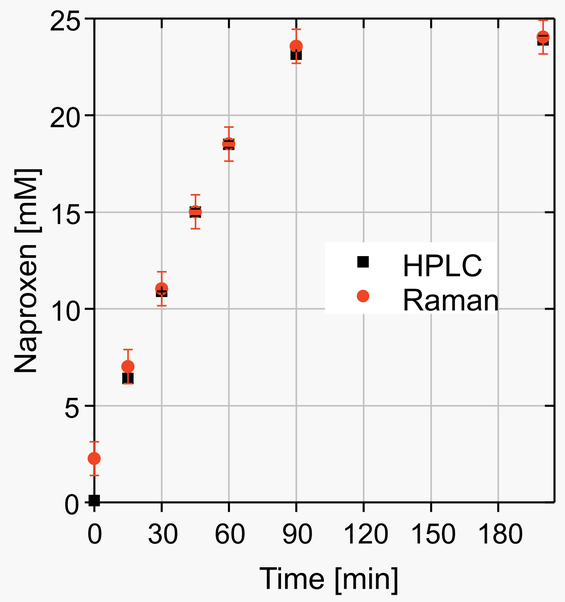
$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n}}$$

PLS model details

# calibration samples	# validation samples	factors	RMSEC	RMSEP
12	7	3	0.2	0.8



Calibration of chemometric model: Predicted vs reference

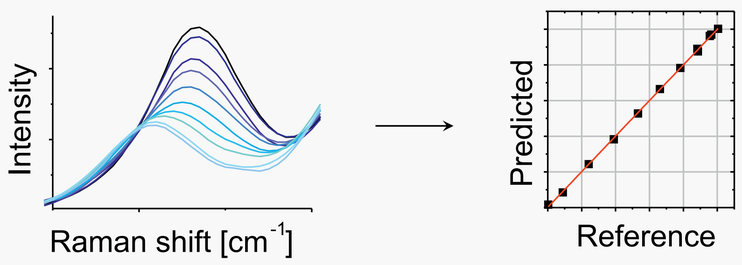


Prediction of reaction progress (independent data set)

Conclusions

- Variety of Raman active vibrations present in biocatalytic naproxen synthesis
- Measurement in aqueaous solution without spectral disturbances caused by water
- PLS model with few calibration samples: low RMSEs, quantification in mM range possible

→ Raman spectroscopy excellent option for in-line monitoring of biocatalytic naproxen synthesis



References:
[1]S. Yoshida et al (2015), Biosc. Biotech. Biochem. 79(12),1965–1971; Assmann et al (2017), Front. Microbiol. 8:448

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