POC4SOC

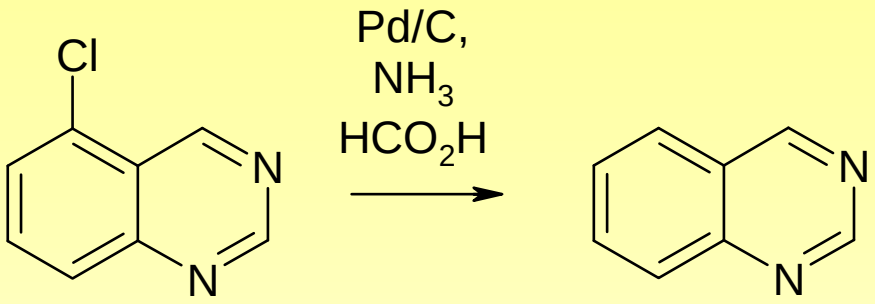
Session 5: Reaction Design (for 1st Order Kinetics)

Pete Shapland

Session Overview

- Practical approach to design of an experiment and initial data analysis.
- Based on extraction of information from off-line HPLC data.
  - Spectroscopic data tends to be related more easily to concentration so manipulation tends to be easier.
  - More scope for introduction of errors.
  - Harder to relate to accurate concentration-time profiles.
- This approach holds for any reaction – not just for (pseudo) 1^{st} order chemistry – and is really simple.

Example - Dechlorination



- Displays first order kinetics with respect to substrate. Active [Pd] is constant.
- Good chromaphore enabling profiling.
  - Example is going to use off-line HPLC analysis.
  - Perceived difficulty in extraction of concentration information from simple response data.

Profiling Objective

We need to generate a reaction time course that is **linearly related to the solution concentrations** of as many components in the reaction as possible.

This involves two aspects:

1. Sampling
2. Analysis

Both of which synthetic chemists routinely do.

We just need to ensure that our sampling and analytical methods can be used to quantitatively relate HPLC peak area to reaction concentration.

Sampling Errors

Sources of error in sampling:

- Sample amount.
- Diluent amount.
- Autosampler injection volume.
- Non-linear response in UV detector.
- Integration errors.

Using constant volume sampling and an internal standard will remove the first 3 sources of error.

Sampling

Sampling must generate samples that are ***stable and representative of the bulk reaction at the time of sampling.***

- We need a consistent sampling technique as this will improve the quality of the data.
- Take samples of **constant volume** using an analytical pipette and quench into constant volume of quench/diluent.
- Constant volume sampling will mean that integration and retention times will be more reproducible and HPLC peak areas should be proportional to solution concentration.

Pipettes

- Two main sorts widely available
  - Aspirated
  - Displacement
- Not cheap but essential kit for off-line analysis.
- Either will work well but think about your chemistry.
  - Near reflux and any pipette will sample vapour but displacement pipettes allow displacement of vapour.
  - Aspirating pipettes are “weaker” for dense and/or viscous phases (eg DCM).
  - Displacement pipettes can be slower to change tips.

Internal Standards

The internal standard can be any component whose solution concentration is constant throughout the reaction.

- Added inert compound (consider response & solubility).
- Solvent (non-volatile).
- Inert impurity in starting material.
- Catalyst.

Very First Reaction

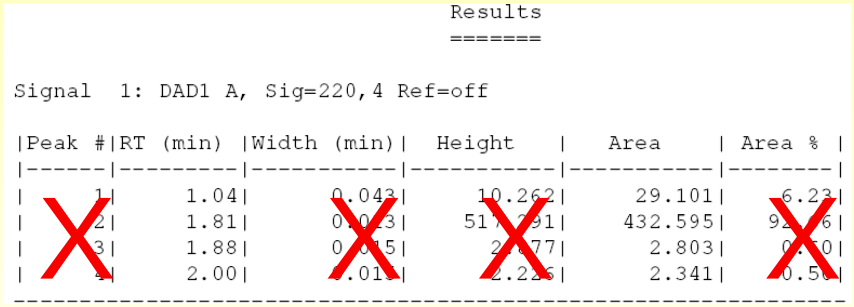
- Not a kinetics study!
- Run standard conditions and take a samples after 5 mins to test potential quenches.
- Analyse repeatedly.
- No need for an internal standard but useful to include.
- This gives 3 pieces of information:
  - Roughly how quick the reaction is (mins or hrs).
  - If the sample taken is quenched.
  - If the sample taken is stable.

First Kinetics Run

- Needs to be **isothermal** if possible.
- Needs to be on reasonable scale.
  - Sampling must not alter the reaction too much
- Use pipettes for sampling.
- Use an internal standard.
- Work out sampling routine.
  - Need more samples at start of reaction rather than at the end
  - Prepare labelled quench mixtures for samples to enable **rapid** and **precise** sampling (include t= 0!)
  - Use a timer for accurate sampling

First Run Data

- A list of peaks and areas that doesn't contain concentration data.



Results
=======

Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.04	0.043	10.262	29.101	6.23
2	1.81	0.043	517.791	432.595	92.66
3	1.88	0.015	2.777	2.803	0.60
4	2.00	0.013	2.226	2.341	0.50

- Most of this is irrelevant.

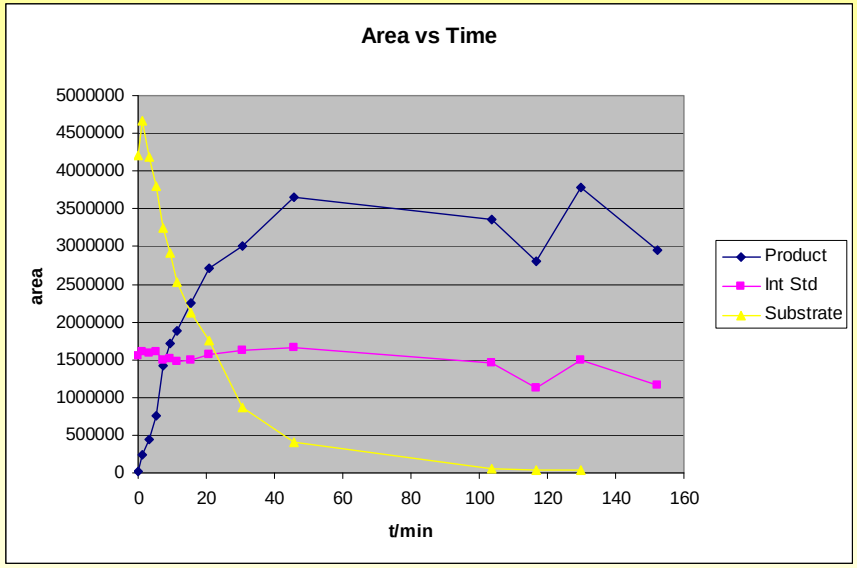
First Run Data

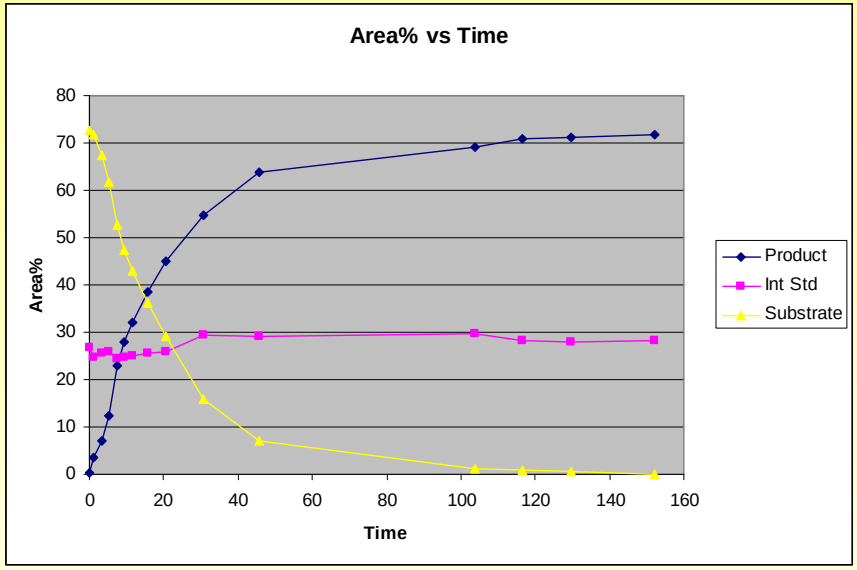
- Arranging all of the samples by time gives:

Time	area			area %		
	Product	Int Std	Substrate	Product	Int Std	Substrate
0	20940	1558519	4215691	0.361337	26.89351	72.74516
1.233333	237655	1598853	4663994	3.655948	24.59584	71.74821
3.25	437126	1583839	4181797	7.04728	25.53442	67.4183
5.266667	755216	1596423	3805131	12.26643	25.92955	61.80401
7.333333	1413906	1498823	3247906	22.95065	24.32903	52.72031
9.533333	1724017	1509782	2912199	28.05105	24.56529	47.38366
11.55	1882937	1470693	2524866	32.03093	25.01818	42.95088
15.56667	2248901	1493787	2113641	38.40121	25.50722	36.09157
20.61667	2703046	1559105	1755913	44.91554	25.90709	29.17737
30.68333	3016169	1621316	869574	54.76914	29.44069	15.79017
45.76667	3655272	1664030	412668	63.76991	29.03068	7.19941
103.8167	3360302	1449132	54296	69.08899	29.79466	1.116345
116.65	2801226	1119732	32046	70.86322	28.3261	0.810675
129.6	3778026	1488560	35595	71.25419	28.07448	0.671328
152.3	2954562	1169625		71.63987	28.36013	0

- This gives a huge amount of information.

First Graphs





- Check for degradation of product.
- Do not get seduced by Area% details.
  - It has no relationship to concentration
  - Remove it from the spreadsheet immediately!
- This data is probably good to get going with first order kinetics assessment but there are a couple of things that can be tackled.

Concentration-Time Data

A simple three step process:

1. Standardise.

   This removes the bulk of the sampling and analysis errors

2. Correct for Relative Responses.

   This gives a truer picture of all species present

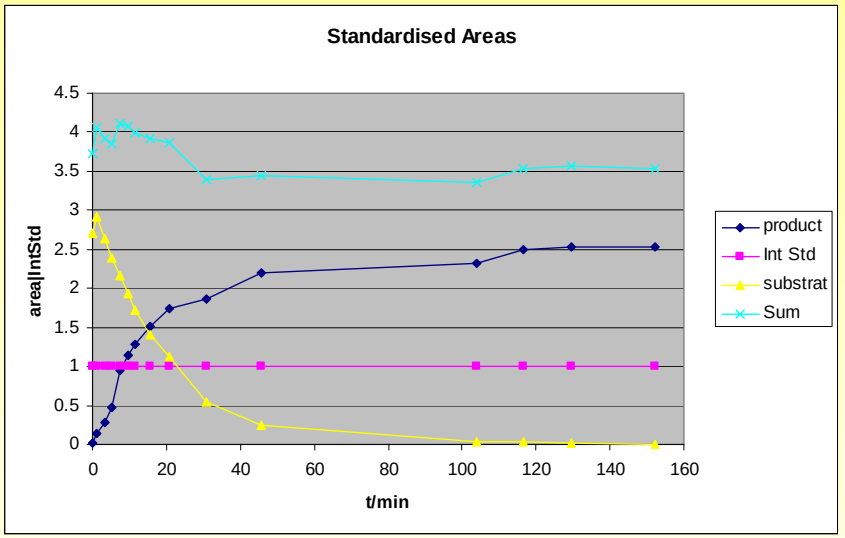
3. Normalise.

   This allows simple multiplication of starting concentration to enable plotting of concentration of all species against time

Standardise

POC4SOC

- Simply divide all areas for each sample by the area of the internal standard for that sample.
- Internal standard becomes a flat line by definition.



- Notice the added line of Sum. This is a check for mass balance and gives valuable information.
- If this line rises, product is absorbing more than substrate
- If this line falls, product is absorbing less than substrate **OR** material is degrading to unobserved products.

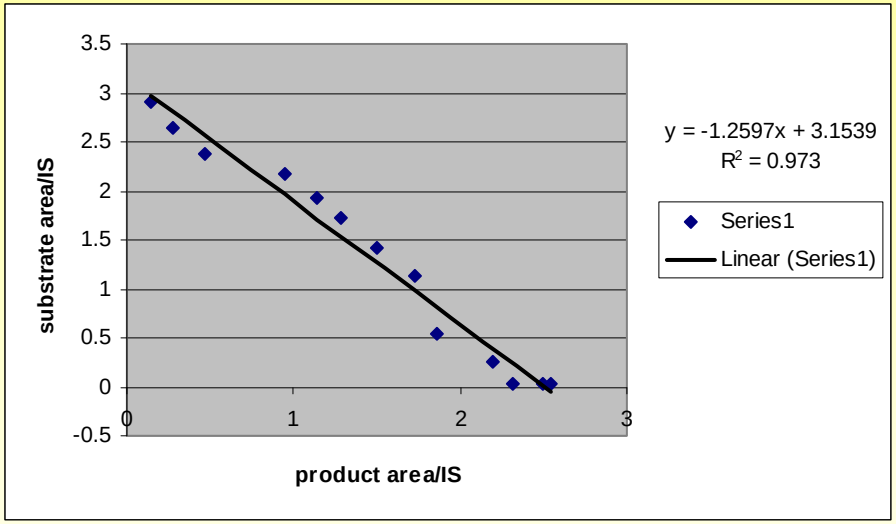
Correct for Relative Responses

- This can be done off-line if authentic, pure samples are available.
  - Make sure that relative absorptions are based on molar equivalents and not weight equivalents.
- It can also be done using the data available.
  - Valuable if a sample is not available.

Calculating Relative Responses

POC4SOC

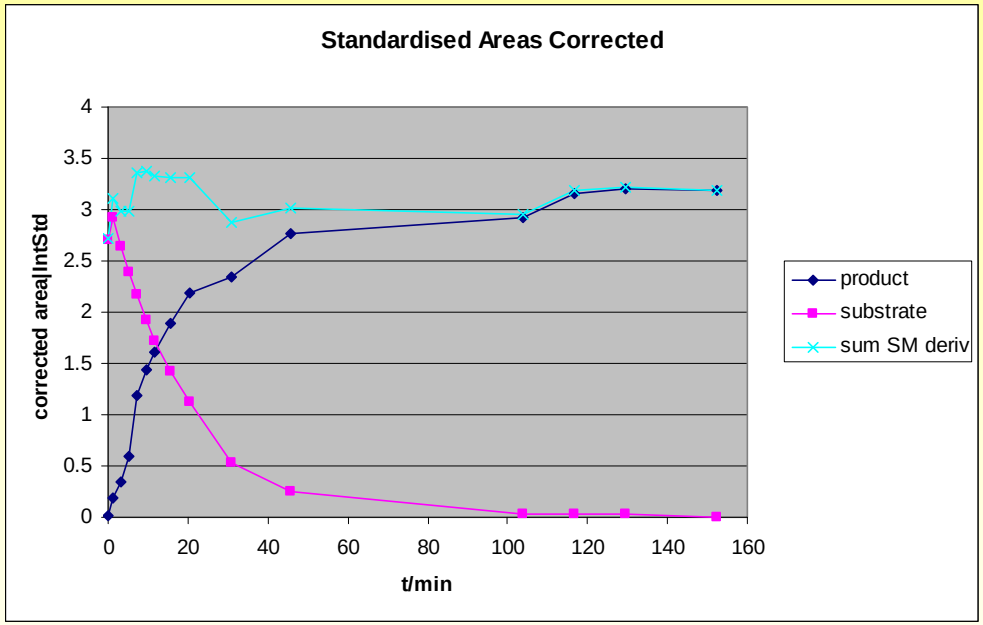
- Plot Area/IS for two of the reaction components.
- If this give a straight line, the slope provides the relative response.
- If this does not give a straight line, then there is some additional pathway (degradation, impurity formation) to account for.



- Normally best to avoid very first and final data points as analysis errors are greatest when peaks are very small.

Correct for Relative Responses

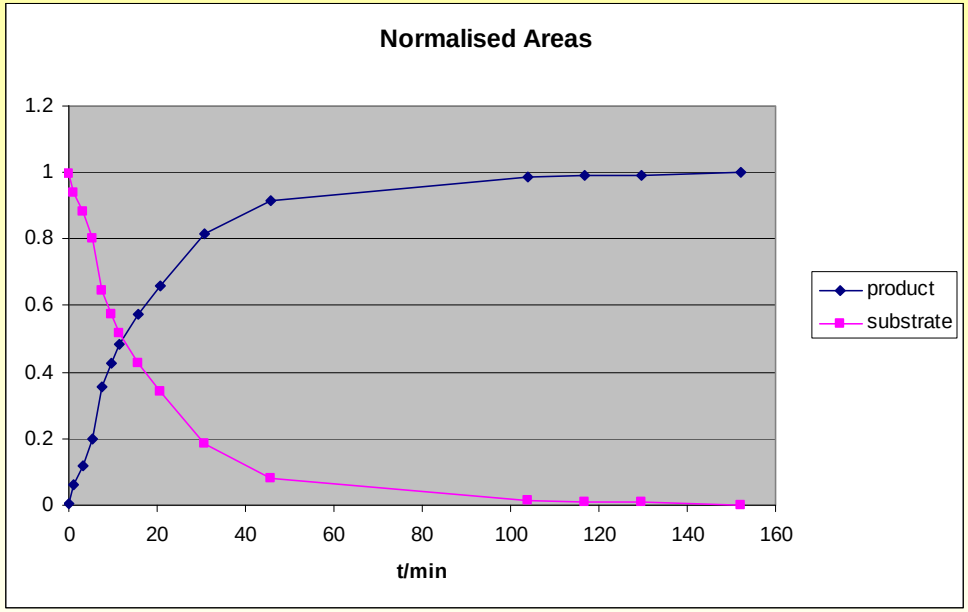
- Note the slight increase in substrate area/IS between first two samples.
- Quite common as solubility can change significantly on addition of a final reagent.



Normalise

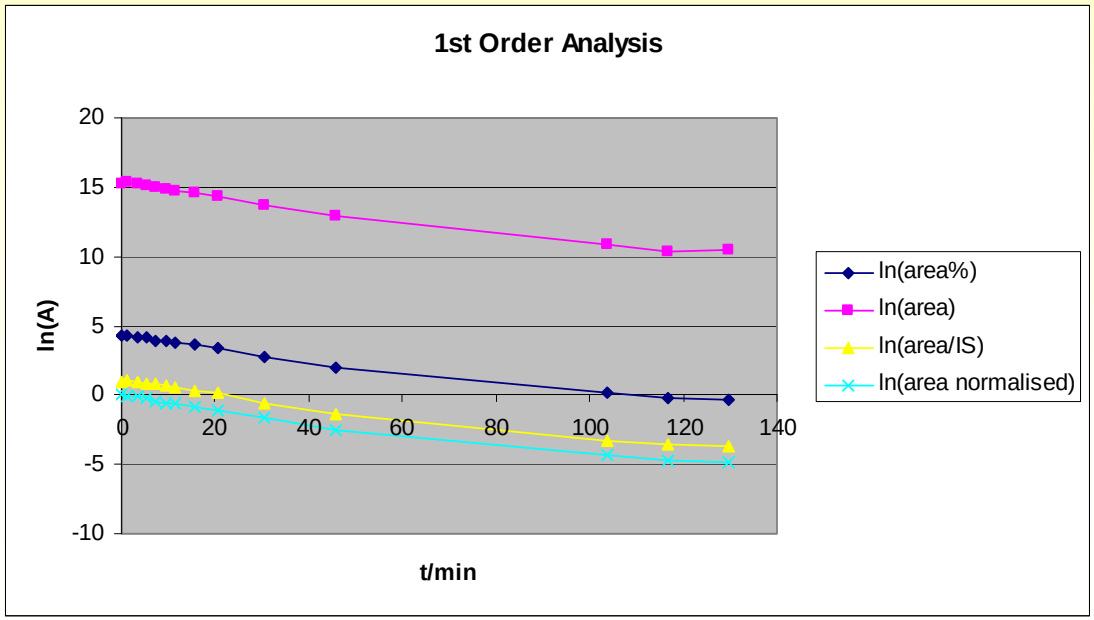
POC4SOC

- Simply means relating everything to the sum of everything derived from substrate.
- This works for a two component system or something with 40 components.
- We can then multiply each data point by the known starting material concentration to obtain molar concentrations for every component for every time point.



1st Order Kinetics

- A plot of ln[A] vs t gives a straight line of slope *k*.
- For 1st order kinetics we can use **any** data set to obtain *k* (=[Pd]*0.039 s^{-1}).
- Any deviation from linearity implies deviation from 1st order behaviour.



Mine Doesn't Look like that!

- Conditions are not sufficiently forcing to ensure 1st Order kinetics across entire reaction course.
- Errors in analysis?
- Errors in reaction set up?
- Reaction isn't 1st Order in component assessed.

What Next

- Examine data thoroughly.
  - Are impurities formed from the start of reaction or from products?
- Use data for further analysis.
  - Computer based modelling, curve fitting, etc
- Run another “identical” reaction at a different agitation rate.
  - This confirms reaction is under kinetic control
- Investigate impact of variation of process parameters.