Enzyme entrapment in calcium alginate beads and kinetic characterization of enzyme immobilized in calcium alginate beads

- Laxman Manjhi 2019BB10034
- Ratnesh Kumar Sharma 2019BB10047

Background

- Immobilization of enzymes by gel entrapment involves trapping the enzyme within a polymeric network.
- Gel entrapment usually does not result in any adverse modification of the enzyme conformation and can provide high yield of immobilization.
- The procedure involves mixing the enzyme with a polymeric solution and forcing the mixture through a fine orifice into a salt solution that insolubilizes the mixture through ion exchange.
- The shape and size of the resulting beads can be controlled by choosing the orifice diameter and the distance of the nozzle from the liquid surface.
- Calcium alginate is a popular polymeric material for entrapment due to its biocompatibility and ability to form stable beads.
- Kinetic characterization of immobilized enzymes involves determining their activity under different substrate concentrations.
- This can be done using a spectrophotometer to measure the absorbance of a reaction mixture containing the enzyme and substrate, followed by calculation of enzyme activity using a standard curve.
- The kinetic parameters of immobilized enzymes, such as Km and Vmax, can be determined and compared to those of the free enzyme to evaluate the effect of immobilization on enzyme function.

Procedure

The enzyme was mixed with the sodium alginate solution in equal volumes to bring the final concentration of sodium alginate to 1% w/v.

The resulting slurry was added drop-wise into chilled calcium chloride solution under gentle continuous stirring at 5-10 rpm using a pump or syringe.

The mixture was allowed to cure in calcium chloride solution for 30 minutes and then washed thoroughly with sodium acetate buffer.

The resulting calcium alginate beads were suspended in acetate buffer.

The diameter and weight of the beads were measured using graph paper and a weighing balance respectively.

For the kinetic characterization of the calcium alginate beads, three different concentrations of sucrose (equal to Km, less than Km, and greater than Km) were prepared from a stock concentration of 1 M sucrose in acetate buffer.

For each sucrose dilution, 5 µL of free or immobilized enzyme was added to 50 µL of freshly diluted sucrose solution.

The mixture was then incubated at 30°C for 5 minutes.

After incubation, 200 µL of alkaline DNS was added to the mixture.

The mixture was then incubated at 90°C for 5 minutes.

Next, 200 µL of 50 mM acetate buffer at pH 4.8 was added to the mixture.

A blank sample (no sucrose) was also prepared and treated with DNS and acetate buffer.

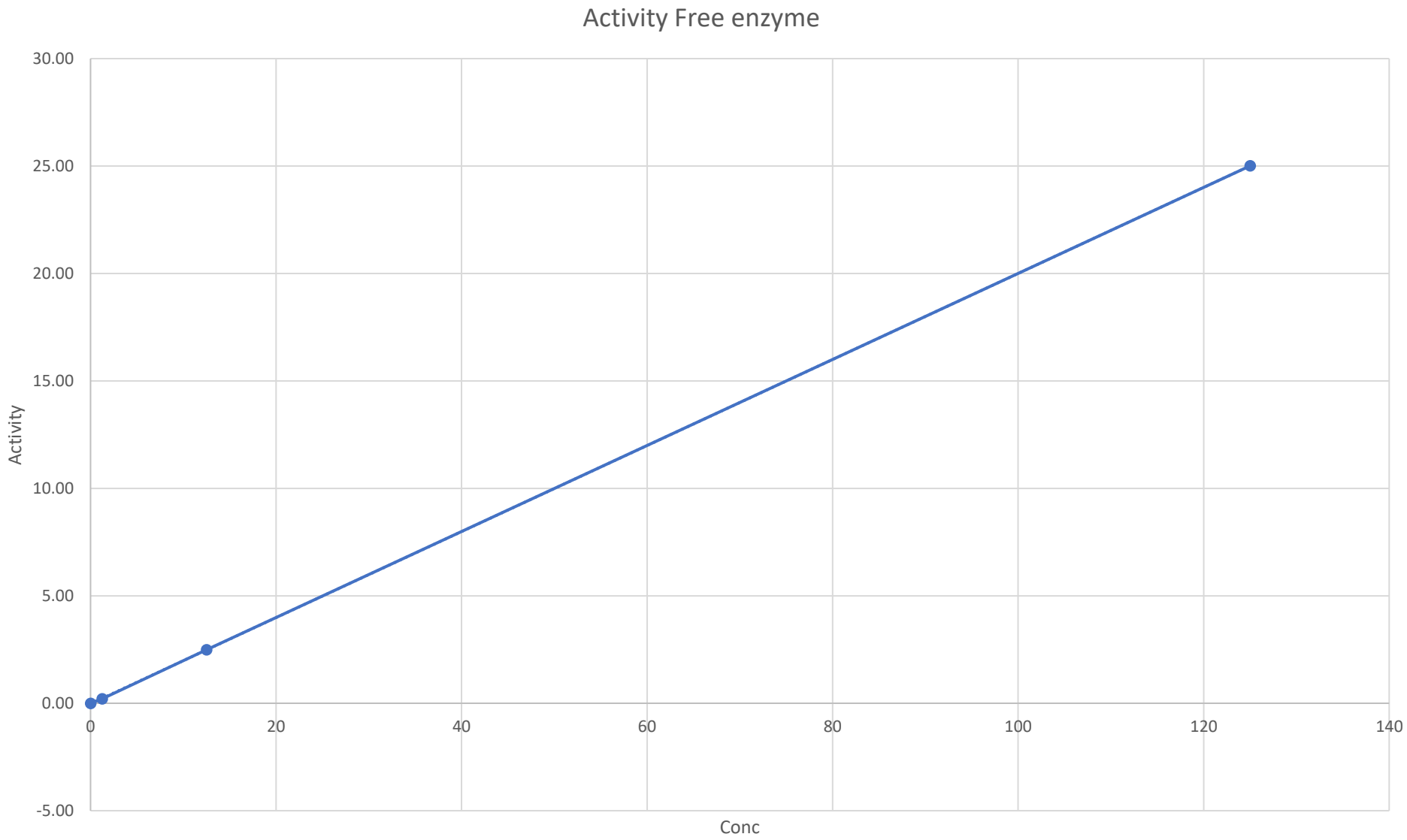
The absorbance readings were recorded at 540 nm for all samples, and the enzyme activity was calculated using a standard curve.

Observation

Free enzyme				Dilution
Blank	<Km	Km (12.5mM)	Km=sat	
0	0.306	0.095	-0.054	10X
Immobilized enzyme				Dilution
Blank	<Km	Km (12.5mM)	Km=sat	
0	0.127	0.223	0.373	10X

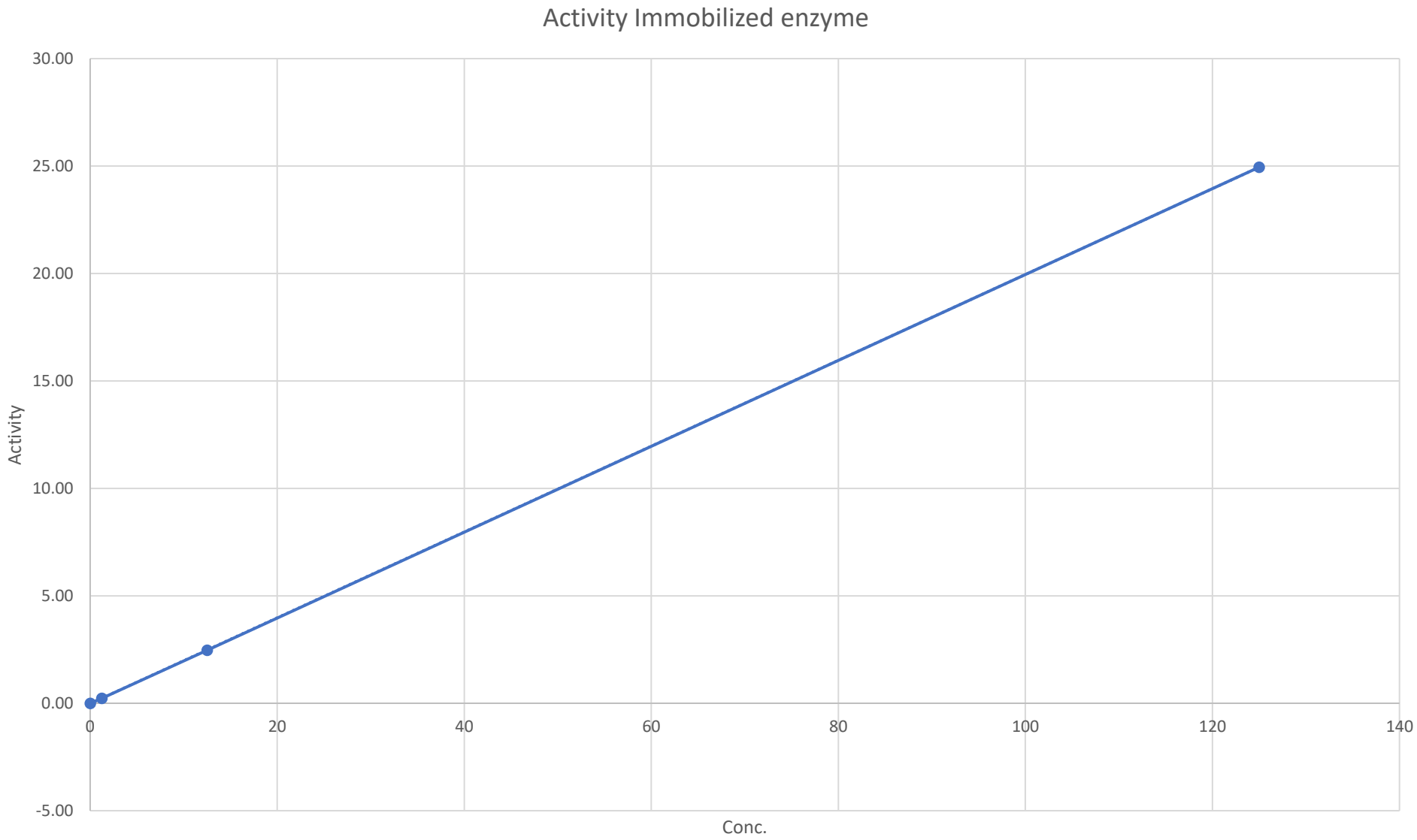
Free enzyme

C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	Activity
0	0	14.2	1	10	0.000	0	5	0.00
1.25	0.306	14.2	1	10	0.215	1	5	0.21
12.5	0.095	14.2	1	10	0.067	12	5	2.49
125	-0.054	14.2	1	10	-0.038	125	5	25.01


Activity Free enzyme
30.00
25.00
20.00
15.00
10.00
5.00
0.00
-5.00
Activity
0
20
40
60
80
100
120
140
Conc

Immobilized enzyme

C1 (mM)	Abs. for C2	ε (mM^-1 cm^-1	l (cm)	Df	C2 (mM)	C1-C1 (mM)	t (min)	Activity
0	0	14.2	1	10	0.000	0	5	0.00
1.25	0.127	14.2	1	10	0.089	1	5	0.23
12.5	0.223	14.2	1	10	0.157	12	5	2.47
125	0.373	14.2	1	10	0.263	125	5	24.95


Activity Immobilized enzyme
30.00
25.00
20.00
15.00
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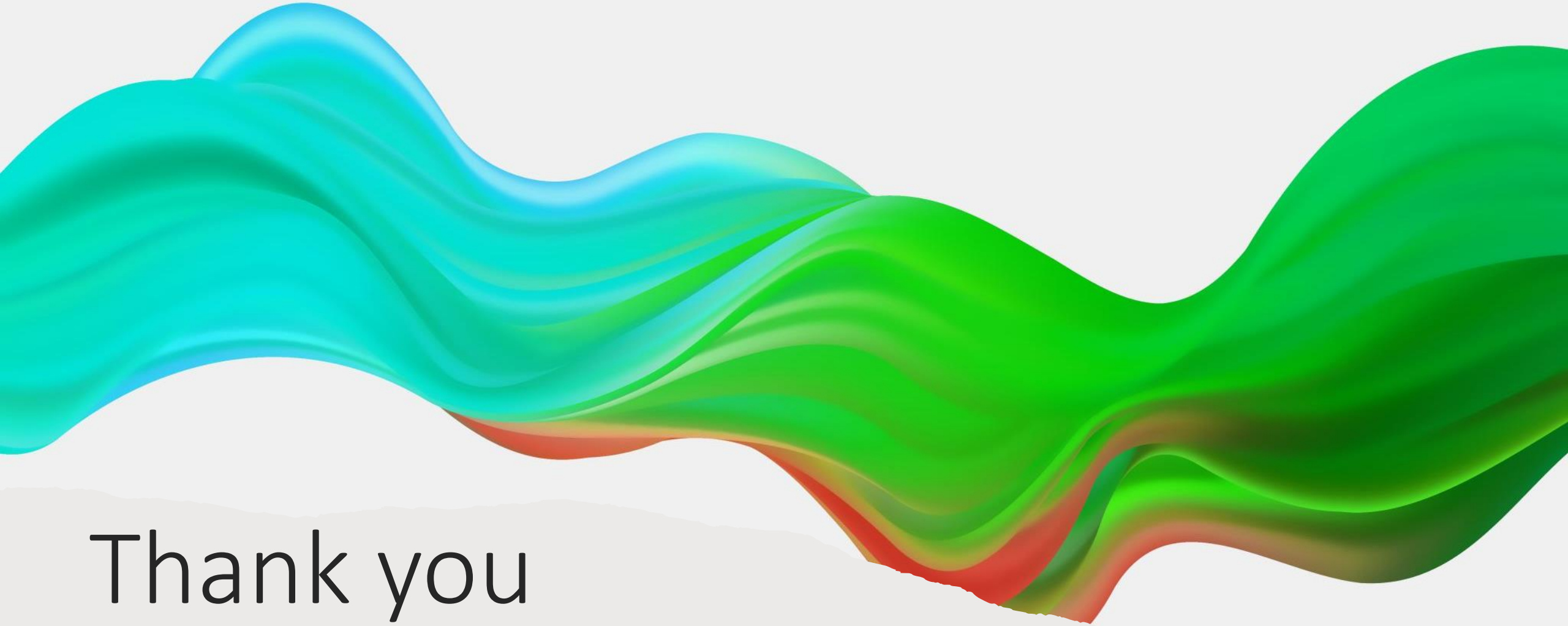
0.00
-5.00
Activity
0
20
40
60
80
100
120
140
Conc.



Thank you