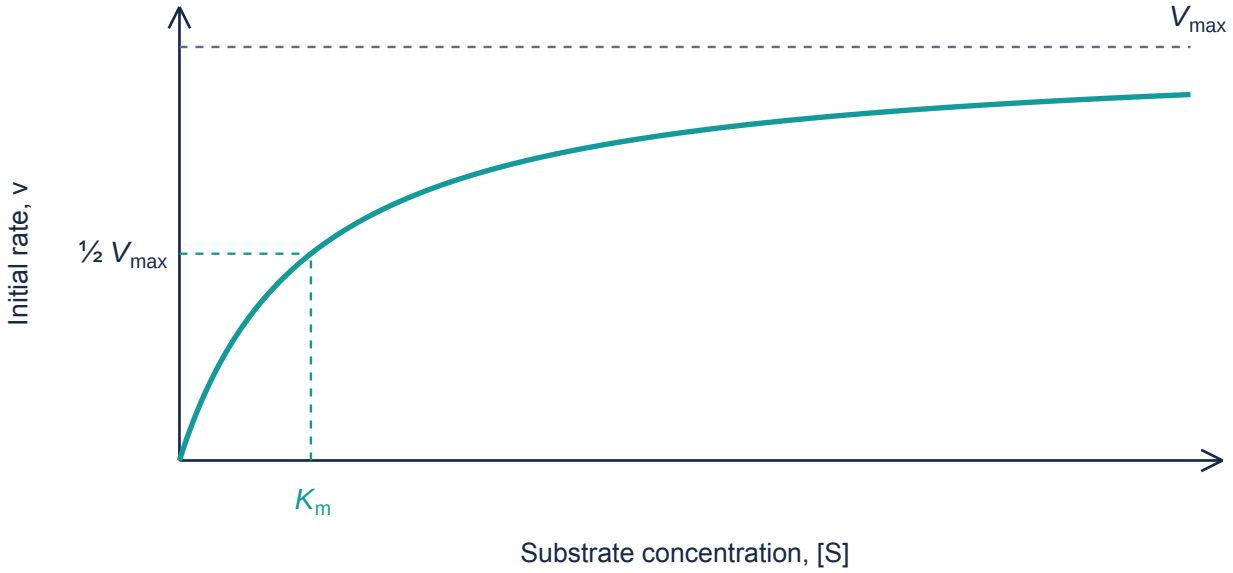
Two values, different meanings

Measure initial reaction rate at a range of substrate concentrations. Keep enzyme concentration, temperature and pH fixed so the curve describes one set of assay conditions.

INITIAL RATE AGAINST SUBSTRATE CONCENTRATION



1. MAXIMUM RATE

V_{max} is the limiting rate approached at very high substrate concentration. Active sites are occupied for almost all of the time, so adding substrate has little further effect. V_{max} has rate units, such as micromoles of product per minute.

2. MICHAELIS-MENTEN CONSTANT

K_m is the substrate concentration at which the initial rate is half V_{max}. Find the half-maximum rate on the vertical axis, go across to the curve, then down to the substrate axis. K_m has concentration units, not rate units.

This is not a product-against-time graph. Its horizontal axis is substrate concentration, and each rate comes from a separate reaction condition.

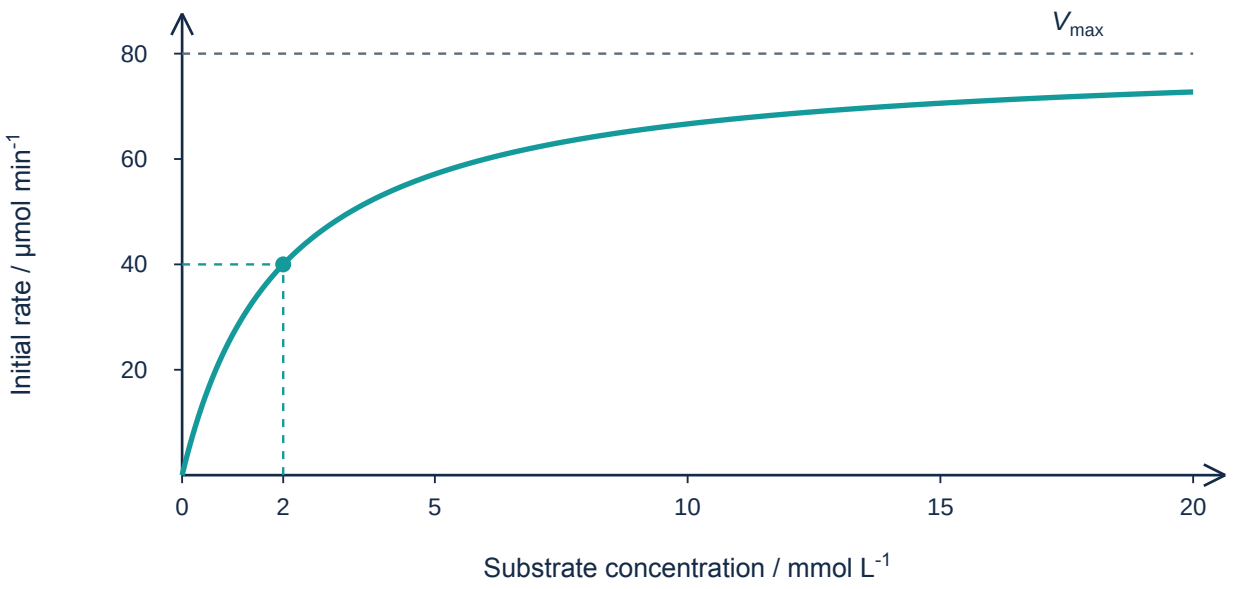
Supporting reading: Worthington Biochemical, Substrate Concentration.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 2, p. 20.

Read the graph step by step

Worked example: the fitted curve approaches a maximum rate of 80 micromoles per minute. Values are illustrative, not experimental measurements.

EXAMPLE: HALF-MAXIMUM RATE IS 40



1. FIND THE MAXIMUM RATE

The horizontal dashed line marks V_{max} = 80 µmol min^{-1}. Use the limiting value, not simply the last plotted rate.

2. HALVE THE RATE

Half V_{max} = 80 / 2 = 40 µmol min^{-1}. This remains a rate on the vertical axis; it is not K_m.

3. READ THE SUBSTRATE CONCENTRATION

At a rate of 40, move horizontally to the curve, then vertically down. The corresponding concentration is 2 mmol L^{-1}, so K_m = 2 mmol L^{-1}.

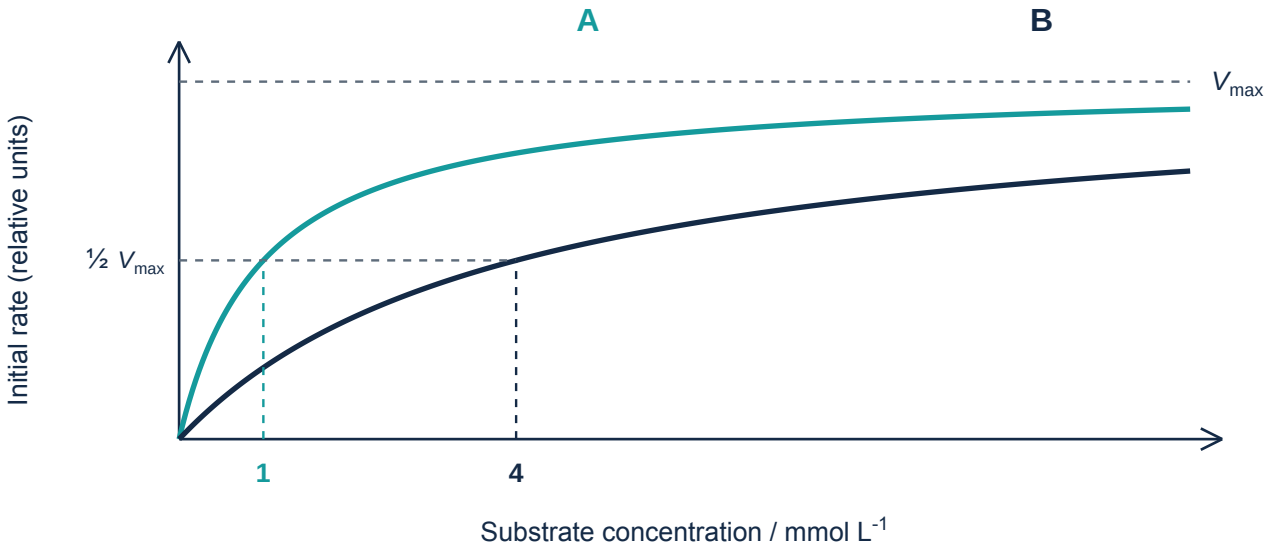
Method background: Assay Guidance Manual, Basics of Enzymatic Assays for HTS.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 2, p. 20.

Compare affinity carefully

In the CIE comparison, a lower K_m usually indicates higher enzyme-substrate affinity: less substrate is needed to reach half of that enzyme's maximum rate.

ILLUSTRATIVE ENZYMES: SAME MAXIMUM RATE



1. LOWER CONCENTRATION AT HALF-MAXIMUM

Enzyme A has K_m = 1 mmol L^{-1}; enzyme B has K_m = 4 mmol L^{-1}. A reaches half-maximum at one-quarter of B's substrate concentration. This is consistent with higher affinity for A in the syllabus interpretation.

2. DO NOT CONFUSE AFFINITY WITH CAPACITY

A higher V_{max} alone does not show higher affinity. V_{max} depends on active enzyme concentration and catalytic turnover. If two curves have different maxima, find half of each curve's own V_{max} before comparing their K_m values.

Scientific nuance: K_m reflects binding and catalytic steps, so it is not always a direct binding-affinity constant. Compare the same substrate under matched conditions; the simple curve assumes Michaelis-Menten behaviour.

Affinity nuance: A Simple Classroom Teaching Technique for Michaelis-Menten Kinetics.
Assay conditions: Assay Guidance Manual, Basics of Enzymatic Assays for HTS.

Scope: Cambridge Biology 9700 syllabus, section 3.2, outcome 2, p. 20.