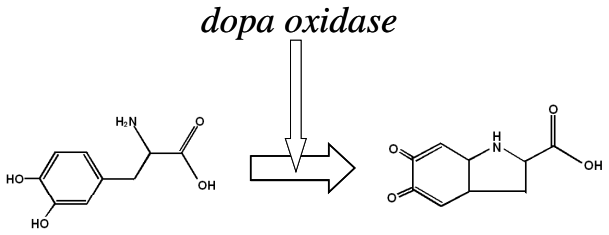
Dopa oxidase

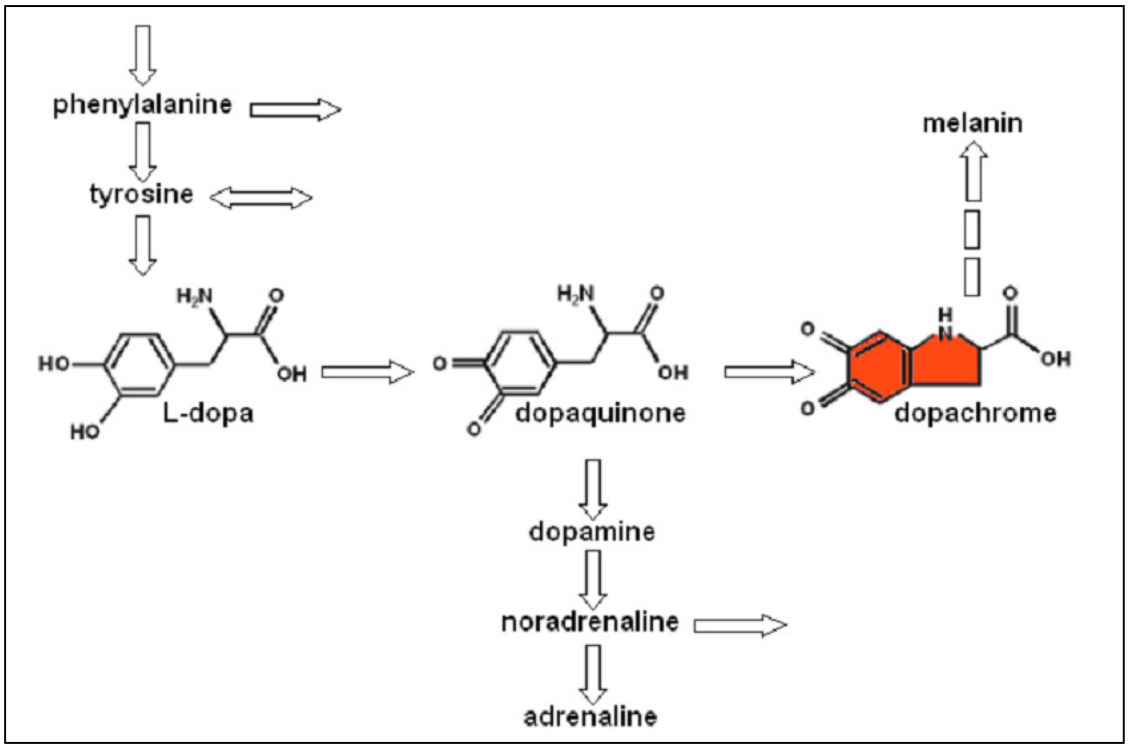
Safety: Good laboratory practice should be followed. There are no specific safety issues associated with L-dopa (which can be bought and taken orally as a dietary supplement) or with the basic method described for extracting and assaying the enzyme.

Reaction: The reaction is the conversion of L-dopa (L-3,4-dihydroxyphenylalanine) to dopachrome (red).



L-3,4-dihydroxyphenylalanine

Dopachrome is what gives red hair its colour.
The reaction is associated with interesting metabolic pathways involved in the formation of melanin, the neurotransmitter dopamine implicated in Parkinson's disease, and the inborn error of metabolism PKU.



Suppliers: L-dopa can be bought from the usual sources such as Sigma-Aldrich but there is a much cheaper alternative.. It is sold as a dietary

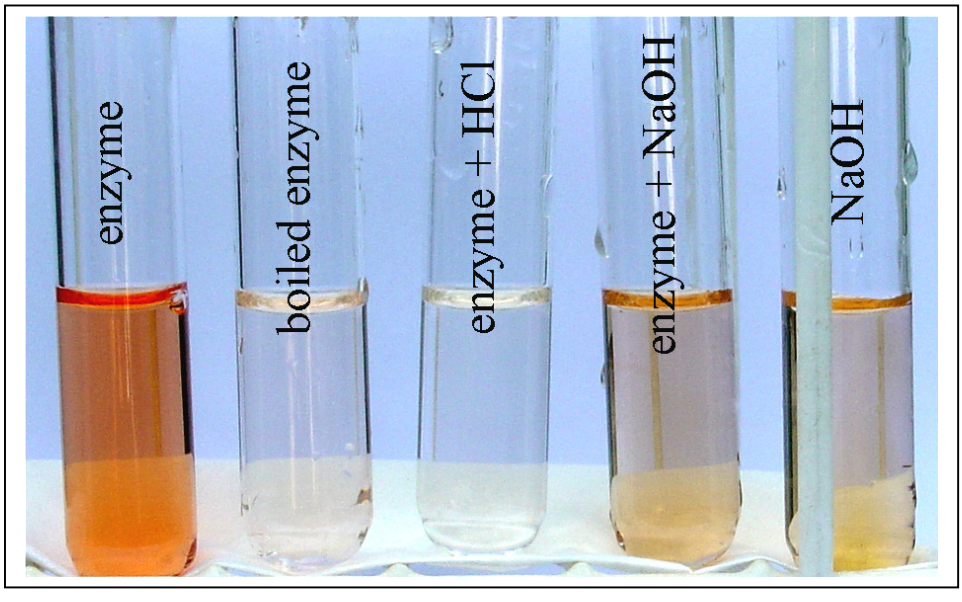
supplement, usually as an extract from the velvet bean *Mucuca pruriens*. Extracts advertised as 98% pure or higher have generally given us results indistinguishable from pure L-dopa at considerably lower cost. A websearch will turn up suppliers and prices around 30p (50¢) per gram. One gram is enough to make about 200cm^3 of saturated solution so costs are minimal.

Concentrations: At 20℃ the limit of solubility of L-dopa is 0.5g in 100cm^3 water. This is 25.4mM (MWt = 197). Solubility is higher in hot water (2.5g/100cm^3 at 100℃) so we would usually make solutions with hot water to speed up dissolving.

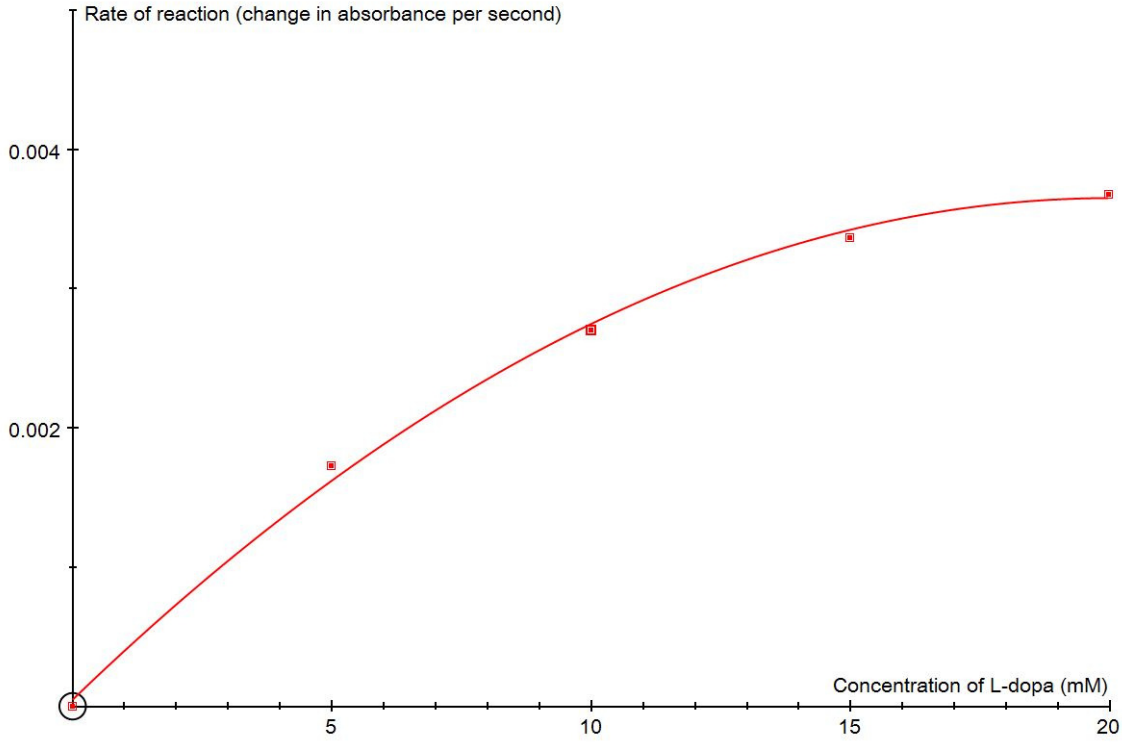
Stability: Dopa solutions do not keep well because there is spontaneous conversion to dopachrome, other intermediates and eventually to melanin. The presence of these intermediates (presumably) effects the rate of the dopa → dopachrome reaction so a solution will give different results when it is reused. Since the cost is low it should not be a problem to make up fresh solution when required.
The spontaneous reactions proceed slowly or not at all in acid so we usually add a drop of acid to aqueous solutions of L-dopa. This needs to be neutralised by adding a small amount of buffer to the assay mixture.

Preparing the enzyme:
Crush about 5g of ripe banana in 20mls water and filter through butter muslin. This will retain its activity for several days, best if refridgerated. Further purification by centrifugation and precipitation with propanone or ammonium sulphate are possible but not necessary for the investigations suggested here.

Basic introduction to some properties of enzymes:
This can easily be done by individual students in a single lesson. Students can prepare the enzyme for themselves and observe the colour change when it is added to L-dopa. There is no reaction if boiled enzyme is added or if a drop of acid is included. In alkaline solution there is a colour change but it can be shown that this occurs in the absence of the enzyme. This is a good illustration of the importance of appropriate controls.



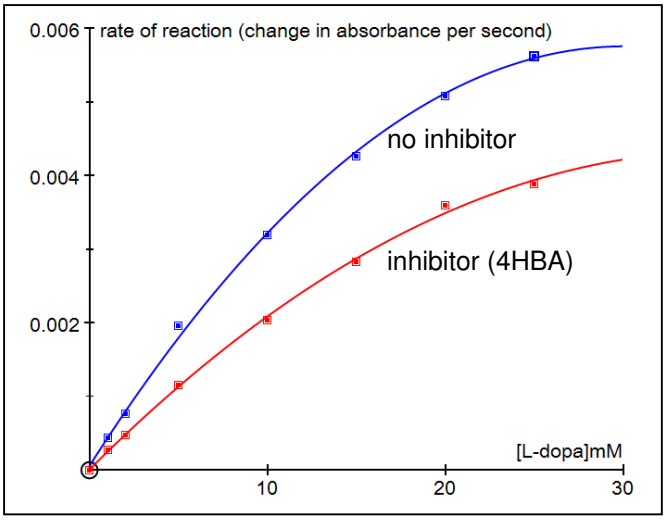
Using the Mystrica colorimeter to measure reaction rates:
Measure the absorbance of blue light. The colorimeter can be used stand-alone or connected to a computer.
It should be easy to obtain initial straight lines to calculate the rate of the reaction.
A suitable reaction mix would be 2cm^3 of L-dopa with 0.1 cm^3 of enzyme extract prepared as above. The concentration of enzyme of L-dopa can be varied to obtain data showing the effect of varying either enzyme or substrate concentration on the rate of the reaction. If the L-dopa solution has had a drop of acid added to prevent the spontaneous reaction then include 0.1cm^3 of buffer (e.g. 1M phosphate buffer pH7).
The reaction can be performed in buffers to investigate the effect of pH but at pHs above 7 the spontaneous reaction will mask the effect on the enzyme.
The graph below shows a typical set of results plotting substrate concentration against rate of reaction.

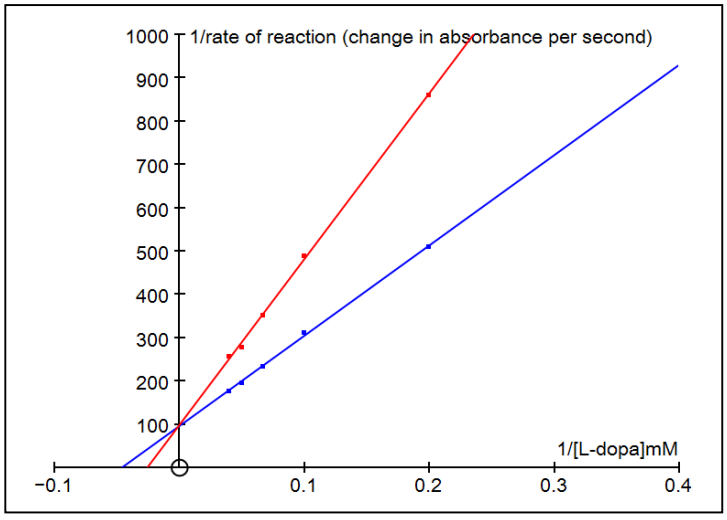


We have found it easily possible for a class of 20 students, working in pairs with a colorimeter for each pair, to extract the enzyme, perform the assay on one concentration, plot the results and obtain a rate ($\Delta A min^{-1}$) in a double period (80mins). With five concentration, each replicated by two groups, a graph similar to the one above can be obtained, (see lesson plans below).

Inhibitors:
There are a number of inhibitors for this reaction. Anything that contains phenols will competitively inhibit the reaction – green tea works well. 4-hydroxy benzoic acid (4HBA, PHBA) is a competitive inhibitor and gives good results as shown in the graphs below. These results were obtained using a reaction mixture of 2cm3 L-dopa, 0.1 enzyme extract and 1 of 2mM 4HBA.

The Lineweaver-Burk plot on the right indicates that the inhibition is competitive.





Practical 1 – Protocol for introducing students to some basics of enzyme studies

Suitable for 11-14 – you could also use boiled enzyme and added alkali (needs control with alkali but no enzyme)

- Take a piece of banana about 1cm long, put it in a plastic petri dish and squash it thoroughly to a paste using the back of a plastic fork.
- Add about 10cm^3 of water and blend to a smooth paste
- Filter through 2 layers of butter muslin into a clean test tube
- Label the tube 'enzyme extract'

- Using the plastic droppers provided set up the three tubes as shown below. **Make sure you add the enzyme LAST**

	Tube		
	A	**B**	**C**
L-dopa solution	3cm^3	3cm^3	3cm^3
Lemon juice	0	0	2 drops
Water	2 drops	0.25cm^3 + 2 drops	0
Enzyme extract	0.25cm^3	0	0.25cm^3

Observe!

Practical 2 Suitable for 15-18

Measure the rate of an enzyme reaction and investigate the effect of changing the concentration of the enzyme.

- Collect
  - Colorimeter
  - Cuvete rack
  - Cuvette
  - Cuvette cap
  - 2 labels
  - Test tube rack
  - Boiling tube
  - 2 small beakers (glass or plastic)
  - 2 pieces of muslin
  - Filter funnel
  - Pestle and mortar
  - 1ml syringe
  - 2ml syringe

- Prepare a table to record Absorbance at ten second intervals for two minutes

- Set a colorimeter to read the Absorbance (A) of blue light (B).

- Collect a little (about 20 cm^3) of L-Dopa solution in a small beaker labelled 'Substrate, L-Dopa)

- Put a piece of banana about 0.5cms long into a mortar with a little sand and grind it to a smooth paste

- Slowly add 20cm^3 of distilled water mixing all the time

- Filter through two layers of muslin into a boiling tube then pour the filtrate into a small beaker labelled 'enzyme'

- Use a 1ml syringe to put 0.5cm^3 of the enzyme extract in a cuvette

- Use a 2ml syringe to add 2 cm^3 of L-dopa solution, immediately cap the cuvette, invert it to mix the contents and put it in the colorimeter

- Press CAL, start the stopwatch and record the absorbance at 10 second intervals for two minutes

- Plot the results on a suitable graph.

- Repeat, varying the concentration of enzyme