Cellulase Method

Scope
- Monitor loss of viscosity of cellulose-thickened materials due to cellulase activity.
- Determine cellulase activity in samples.
- Quality control.

Rapid Visco Analyser
The Rapid Visco Analyser (RVA) is a cooking stirring viscometer with ramped temperature and variable shear profiles optimized for testing viscous properties. The instrument includes international standard methods as well as full flexibility for customer tailor-made profiles. Combining speed, precision, flexibility and automation, the RVA is a unique tool for product development, quality and process control and quality assurance.

Description
Although commonly used to detect $\alpha$-amylase activity, the RVA can also be used to detect the activity of other polysaccharide endohydrolases including the endoglucanases found in the cellulase group of enzymes. Cellulose-based products are commonly used to thicken paint and some foods. Contamination with cellulase (e.g. from bacteria) can cause unacceptable viscosity loss in the product after production. Loss in viscosity can be measured in the RVA using a substrate such as Na$^+$ - carboxymethyl cellulose (CMC), and related to the cellulase activity.

![Figure 1](image1.png)

Fig. 1. Loss of viscosity as a function of cellulase activity, using fungal cellulase of known activity from *Aspergillus niger*. Viscosity Loss Rate (RVU/min) = 9.2 x (Cellulase Activity) + 0.5. Note the loss of sensitivity in the test above 1.2 EU.
Method
Thirteen-minute pasting profile.

Sample Preparation
4.00 g sample (e.g. ground meal) or 4.0 ml suspension (e.g. paint) and 25.0 ml CMC Citrate Buffer.

Preparation of CMC Citrate Buffer
Prepare 1 L of 0.005 M sodium citrate buffer, pH 4.8, and thoroughly mix into it 2% w/v Na\(^+\) – CMC (Sigma, high viscosity). Pre-heat solution to 45°C using a water bath or similar apparatus.

Profile

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<tbody>
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</tr>
<tr>
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<td>Speed</td>
<td>160 rpm</td>
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<tr>
<td>00:10:00</td>
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Idle Temperature: 45 ± 1°C
Time Between Readings: 4 s

Measure
V1: Viscosity at 1 min. (°C)
FV: Final viscosity (cP)

Calculate the rate of loss of viscosity as FV minus V1 (RVU/min). This is the RVA Cellulase Index.
Cellulase activity in EU can be calculated from a standard curve.

The assay is sensitive for cellulase activities between 0.1 and 1.2 EU. If activities exceed these limits, alter the concentration or amount of sample as appropriate and repeat the assay.

To determine cellulase activity in EU, a standard curve can be generated using the above substrate and testing various amounts of cellulase of known activity (obtainable from e.g. Sigma Chemicals). An example of a standard curve is given in Fig.1.