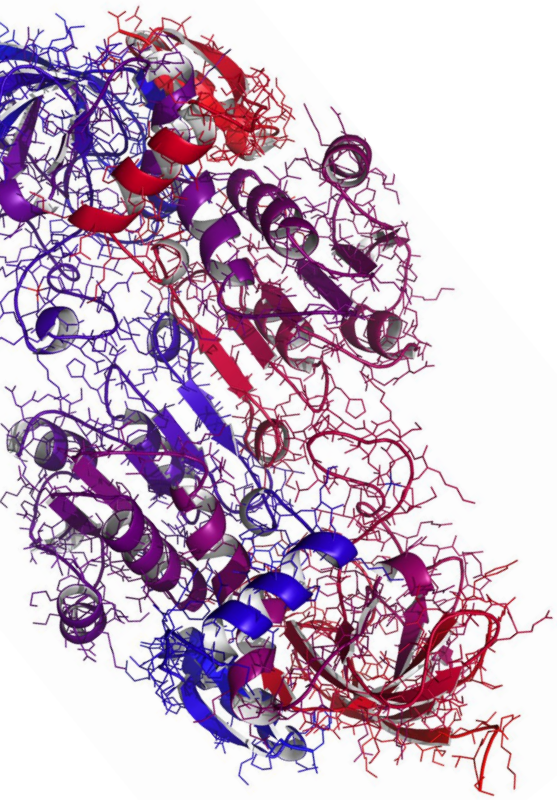
Smart Notes

Standardization of routine enzyme activity for product consistency

Enzyme activity is a measure of the active enzyme quantity and is affected by a variety of factors, such as temperature, pH, and concentration. Enzyme activity assays are critical to test for product consistency since it provides a metric of the exact amount of enzyme used to manufacture the product. Although enzymes are widely used in industries, their activity assays using spectrophotometry are difficult to perform in an industrial setting due to a lack of standardized spectrophotometric assays, which could provide high reproducibility and convenient operations. By standardizing these assays, the enzyme activity could be compared between different samples and across different labs to establish consistency in industries such as pharmaceutics, medical diagnostics, food and beverage processing, animal nutrition, textiles, household cleaning products, automotive fuel and energy generation.



How can enzyme activity assays be standardized in an industrial setting using UV-Vis spectrophotometry?

The calibration for enzyme activity assays was standardized by Thermo Scientific™ Evolution 350 UV-Visible Spectrophotometer (EVO 350) using Amplex® Red Glucose/Glucose Oxidase Assay Kit. Glucose oxidase (GOx) was used as a test enzyme. In the assay Kit, GOx reacts with d-glucose to form hydrogen peroxide, which in the presence of horseradish peroxidase (HRP) reacts with the Amplex® Red reagent to generate an oxidation product, which is accurately detected by EVO 350 at 560 nm. EVO 350 includes Thermo Scientific™ INSIGHT™ Software that provides ease of programing new methods and guides through each step of analysis by collecting and processing data, as well as printing reports. For industries that need 21 CFR Part 11 compliance, the software has an optional security suite that enforces data security and facilitates end-to-end traceability of samples and associated laboratory processes to provide a central repository for data and test results, ensuring regulatory and pharmacopoeias (USP, EP and JP) compliance.

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Materials needed

The Evolution 350 UV-Visible Spectrophotometer includes INSIGHT Software with an optional security suite, Amplex® Red Glucose/Glucose Oxidase Assay Kit, and disposable semi-micro plastic 10 mm cuvettes.

Procedure

1. **Assay:** Follow the procedure in the Amplex® Red Glucose/Glucose Oxidase Assay Kit to complete the enzyme assay. In brief, fill each cuvette with 200µl of four standard GOx solutions (0.5, 1.0, 2.0, 5.0 mU/mL) and control (assay buffer). Then, to each cuvette, add 200 µL working solution containing a mixture of 100 µM Amplex® Red reagent, 0.2 U/mL HRP, and 100 mM glucose. The procedure to prepare this working solution is described in the kit. For blank measurements fill one cuvette with only assay buffer.

2. **Set parameters in INSIGHT security:** Figure 1 shows how to make a new method for the GOx assay in the INSIGHT Software. Select Quant and standard curve. Define the following parameters: analysis wavelength - 560 nm, integration time – 1 sec, bandwidth – 1nm and define your standard GOx concentration range.

3. **Measurement:** Select “Measure” in the upper portion of the application home page. The program will first prompt the user to run the blank before measuring all listed GOx standard solutions.

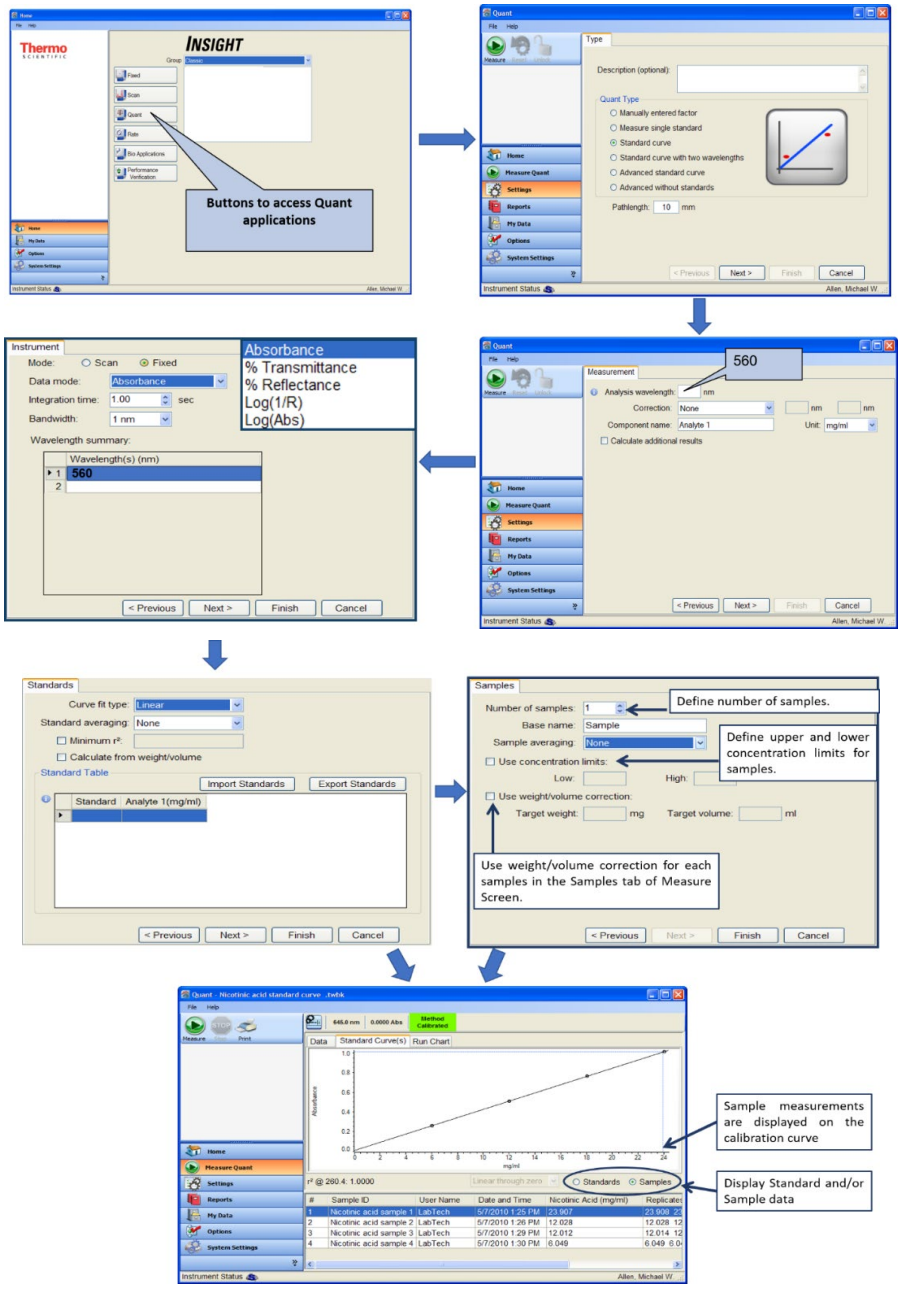

Figure 1. INSIGHT Software guides to setup new standard calibration in Quant method.

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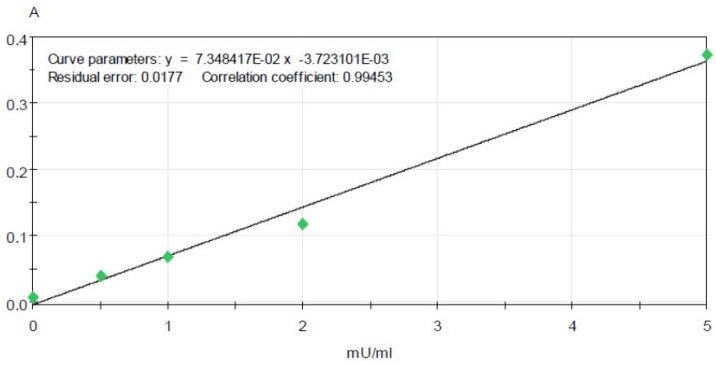
Results

The calibration for enzyme activity assay was standardized by EVO 350 using Amplex® Red Glucose/Glucose Oxidase Assay Kit. Figure 2 shows a linear calibration plot with a dynamic range of 0.5 to 5 mU/mL glucose oxidase enzyme. This calibration curve can be used to measure the exact amount of active enzyme present in unknown GOx samples. The absorbance values of the sample will correlate to the related concentration and display on the calibration as shown in Figure 2. The program also allows users to define sample lists including limits like weight/volume correction and upper/lower concentrations. Similarly, calibration methods can be developed for other enzyme assays such as uric acid, alkaline phosphate assay, and creatinine assay. In our procedure, GOx assays were performed at room temperature. For some assays that need higher temperature for incubation, Thermo Fisher Scientific provides a Peltier accessory with smart linear 8 cell changer (Figure 3) that is compatible with the Evolution 350 spectrophotometer.



Standards

No.	Concentration [mU/ml]	Ordinate [A]	Error [A]	Used
1	0.00	0.007	0.010	Yes
2	0.50	0.040	0.007	Yes
3	1.00	0.068	-0.002	Yes
4	2.00	0.117	-0.026	Yes
5	5.00	0.374	0.010	Yes

Figure 2. Linear calibration plot with a dynamic range of 0.5 to 5 mU/ml glucose oxidase enzyme.

Figure 3. The 8-Cell Peltier system for Evolution Spectrophotometers.