

# Advancing Enzyme Analysis

Fully automated enzyme analysis can ease a persistent bottleneck in biocatalyst development for bio/pharmaceutical applications.

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**E**nzymes—the biological catalysts that accelerate chemical reactions inside cells—play crucial roles in every living organism. The catalytic activity of these protein molecules is widely recognized, speeding up reactions with high specificity and efficiency under mild conditions.

Over recent decades, the use of biocatalysts has rapidly expanded across multiple industrial processes. These enzymes are divided into six classes based on the types of reactions they catalyze—hydrolases, oxidoreductases, transferases, lyases, isomerases, and ligases/synthetases. They are used in the form of cell lysates, whole cells, or purified enzymes, prepared either from their native cells or using recombinant technology in alternate host cells.

Enzyme-based catalysis can offer significant advantages over the use of conventional chemical catalysts. Biocatalysis not only helps speed up manufacturing processes but can also improve quality, reduce waste, and optimize product yield, ensuring greater cost-efficiency and increased profitability.

In the biopharmaceutical and pharmaceutical industries, enzymes are fast becoming the catalyst of choice for the development of new drugs, vaccines, and diagnostic kits. Their potential advantages include exceptional specificity towards recognized substrates, simplified drug synthesis routes, and providing an opportunity for a more sustainable and consumer-friendly approach by replacing harsh chemicals typically used in the vaccine production process.

Despite the clear benefits of biocatalysis, the ability to identify, obtain, test, and optimize enzymes with suitable characteristics for specific applications remains challenging.

Advances in screening technologies and bioinformatics are fueling the rapid discovery of new enzyme variants. The application of protein engineering supported by computer modeling is also expanding opportunities to modify the structure of an enzyme to try to improve its properties.

Every candidate enzyme will work best under a specific set of conditions, which need to be carefully defined by R&D laboratories. But traditional assays used to assess enzymatic activity have significant limitations. With increased demand for the use of enzymes within bio/pharmaceutical production, there is a need for accurate and streamlined enzyme activity and enzyme kinetics analysis.

## THE IMPORTANCE OF ENZYME ANALYSIS

Enzyme assays are laboratory methods for measuring enzymatic activity and are vital for the study of enzyme kinetics

and enzyme inhibition. Activity is monitored by measuring the decrease of substrates, or increase in products, over a defined amount of time. Usually, the assay is carried out by determining the enzyme activity with, and without, activation by an added coenzyme.

Enzymes work by binding to their specific substrates and forming an enzyme-substrate complex. Within the specific reaction, the substrate is converted into the product(s) which is/are then released. Enzymatic activity measures the rate of an enzyme reaction and is generally defined by the amount of substrate converted (or product formed) in a specific amount of time.

Enzyme kinetics describe the speed at which an enzyme-catalyzed chemical reaction proceeds. The speed of the reaction depends on the amount of enzyme used as well as the amount of substrate. While speed increases proportionally to the enzyme concentration, adding more substrate will initially increase speed linearly; eventually, however, the reaction reaches a maximum speed.

Most enzyme assays are traditionally based on spectroscopic techniques, with absorbance and Quorescence the most used types. Due to its low cost and relative simplicity, the spectrophotometric assay remains the most widely used method (1). This classic enzyme test involves following the course of a reaction by measuring the changes in the intensity of light absorbed or scattered by the solution.

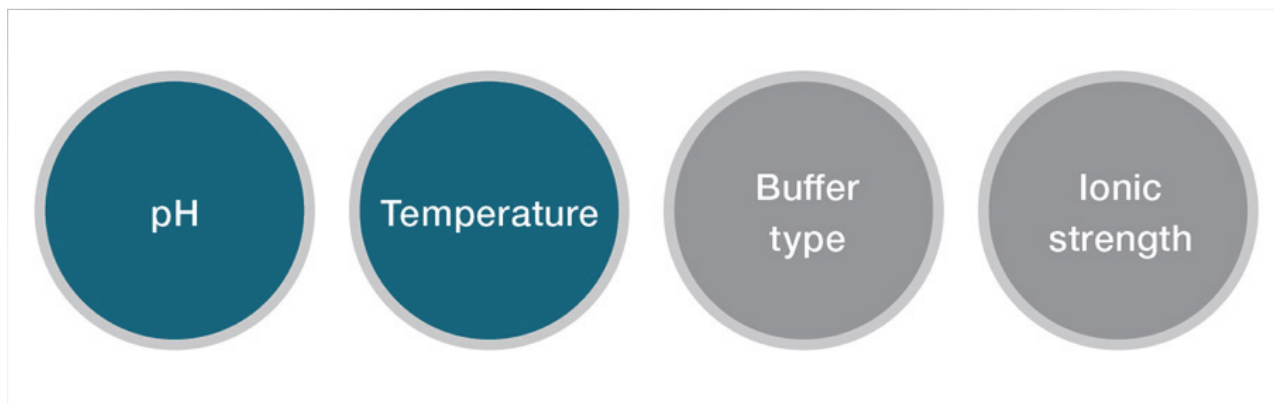
Enzyme assays based on photometry, Quorometry, 96-, 384-, or even 1536-well microplate formats offer a high-throughput alternative to traditional spectrophotometers. However, these methods have limited incubation temperature, temperature stability, and precision due to edging effect, limiting their range of applications.

## THE CHALLENGES OF ENZYME ANALYSIS

Measuring enzyme activity is a precise job and can be influenced by many variables, such as temperature, pH, buffer type, or ionic strength (**Figure 1**). Ensuring all these variables are fixed is critical for achieving reproducible results in enzyme assays. For instance, the accuracy of the results is highly dependent on temperature stability. Just a one-degree change in temperature can lead to a 4–8% variation in enzyme activity. Adding the sample, buffer, and substrate in a specific sequence and time interval is also critical for achieving accurate and reliable results.

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**Figure 1.** Examples of variables that can influence the measurement of enzyme activity. All figures are courtesy of the author.



The major disadvantage of traditional spectroscopic techniques is that all steps involved are manual and can lead to inconsistent results. Based on the enzyme type, the process could take 30 to 60 minutes per enzyme from sample start to data collection, which severely limits throughput. These methods are only suitable when analyzing a few samples or carrying out routine tasks.

While the microplate format approach offers a convenient alternative for high-throughput analysis, this method suffers from temperature stabilization, absorption correction, and edge effects. As the absorbance is measured vertically on microplates through the well, several factors affect the liquid pathlength, and, therefore, the absorbance. As a result, in photometric microplate measurements, a pathlength correction is required for calculating the enzyme assay, and the primary cause of the “edge effect” phenomenon, which is commonly associated with 96-well plates, is attributed to an increased evaporation rate in the circumferential wells compared to those in central locations.

For some enzyme assays, it is necessary to quench or stop the reaction at a specific time to prevent further generation of the product. For example, samples may be collected every five minutes for a predetermined amount of time with the product then measured using high-performance liquid chromatogra-

phy (HPLC), with each analysis taking 30 minutes to complete.

Developing a reliable analytical method for an enzyme assay involves many steps and often requires extensive work (**Figure 2**). Overall method development is laborious and time-consuming, taking four to six weeks even for highly-trained personnel. The process starts with identifying the key method variables, which requires the testing of many sets of samples to determine the optimal experimental conditions. There is a clear need for a new method utilizing automated solutions that can overcome the key challenges associated with traditional approaches for enzyme analysis.

### **STREAMLINING ENZYME ANALYSES WITH DISCRETE ANALYZER TECHNOLOGY**

The introduction of innovative and dedicated discrete analyzers offers opportunities to streamline and simplify enzyme analyses. These dedicated discrete analyzers (enzyme analyzers) can efficiently manage the simultaneous analysis of many different enzymes and measuring conditions in a single instrument that offers consistent and reliable performance. All the necessary enzyme analysis steps are automated, which helps to control the variables and makes the overall method development easy and repeatable.

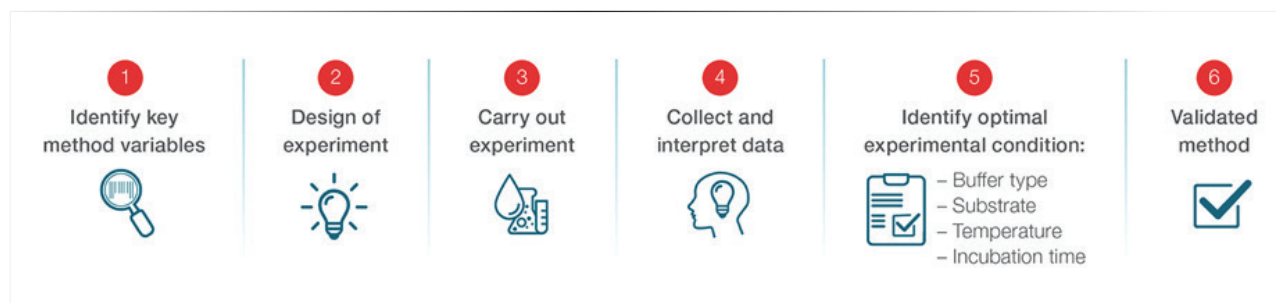
Fully automated enzyme analyzers offer several useful advantages over

more traditional methods for enzyme assays. First and foremost, these instruments provide the opportunity to fully automate the critical steps involved in reliable enzyme analysis. For consistent results, an assay should be carried out in well-defined conditions that can be duplicated in other laboratories. Precise temperature control within  $\pm 0.3$  °C eliminates the risk of sample overheating along with accurate incubation time. Superior temperature control, along with the lack of edge effects, can assure confidence in results. Precise liquid handling (sample, buffer, and substrate addition and mixing) can also improve reproducibility between experiments without compromising on throughput (2).

The technology also enables increased productivity. Within the instrument, samples are simultaneously prepared and queued, delivering one set of results after another. This makes it possible to evaluate many different enzymes, substrates, and methods more quickly and easily (2).

A fully-automated workflow also enables simpler, faster overall method development. Once the testing workflow is set up, the instrument can be left unattended, freeing up time for laboratory staff to carry out other important tasks. Thanks to dedicated software that accompanies enzyme analyzers, workflows are simple and there is practically no changeover time from one method to another. Data traceability is supported by time-stamped raw data, audit trails, user

**Figure 2.** The six steps of enzyme assay method development.



administration and confident certificate of analysis (CoA).

Flexible method parameters for each enzyme type—measuring wavelength, blank measurement, buffer addition, reagent additions, substrate addition, enzyme-specific incubation temperature and time, and data collection duration—allow enzyme assay development and transfer from R&D to quality control laboratories.

And finally, unlike spectrophotometric and microplate methods, a dedicated enzyme analyzer can offer a wide range of incubation temperatures, from 25 °C to 60 °C, expanding the scope of potential applications (3).

## CONCLUSION

For many bio/pharmaceutical manufacturers, there are benefits to be gained from replacing chemicals with enzymes to catalyze reactions. Biocatalysis adheres to green chemistry principles, offering an attractive option for cost-efficient, sustainable manufacturing for processes such as drug synthesis and vaccine development.

An enzyme analyzer based on discrete analyzer technologies offers significant advantages for enzyme research and analysis, including walkaway efficiency combined with faster, easier, and reproducible method development, which leads to better results. These

instruments can help fulfill the growing demand for automation and digitization while overcoming the challenges associated with traditional enzyme assays.

The use of accurate, streamlined enzyme activity and kinetics analysis will pave the way for the widespread adoption of biocatalysis in the development of new pharmaceutical drugs, vaccines, and diagnostics kits.

## REFERENCES

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