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How to extend range linearity in enzyme inhibition-based biosensing assays

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Abstract

Bioassays based on enzyme inhibition are analytical tools widely employed for inhibitor analysis. Beside the conventional analytical techniques such as chromatography and mass spectrometry, these bioassays are cost-effective, easy to use, and suitable for in situ measurement but they are often characterised by a quite narrow linear range.

Herein, we reported a novel graphical method based on integrated Michaelis-Menten equation, valid for all types of reversible inhibition, which provides an extended linear range. The suitability of this innovative approach was demonstrated in the case of fluoride quantification using a colorimetric bioassay based on acetylcholinesterase inhibition. The "half time reaction", estimated by the progress curve of cholinesterase inhibition, was plotted versus the fluoride inhibitor concentration, observing an extended linear range up to 5 mM, instead of 0.6 mM using initial rate measurements. The applicability of this new concept was further demonstrated in the case of catalase enzyme inhibited by cyanide.

Furthermore, it was demonstrated that fixed substrate conversion at level of 10-50% allows determination of inhibitor concentration in a wide linear range with high precision and in short time of analysis. This novel theoretical and practical approach allows the extension of the linear range without any further experiments, with several advantages including low reagent consumption, reduced waste generation and time of measurement.