Review of: "Determining kinetics parameters of bovine serum albumin-protected gold nanozymes toward different substrates"

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Potential competing interests: No potential competing interests to declare.

Review of

„Determining kinetics parameters of bovine serum albumin protected gold nanozymes toward different substrates“

by Saeed Reza Hormozi Jangi

It is difficult to asses the importance of this work because of deficiencies in the presentation. These are broadly nomenclature, experimental detail, and evaluation of the results. I suggest a more careful presentation.

Nonemclature. The title and text refer to nanozymes and the text makes comparison with enzymes. The word is misleading. „Enzymes“ was coined from the Greek and zymos refers to leavening and fermenting. This root refers to biological processes, and enzymes are biological products. This work refers to an inorganic catalyst, and in no sense is the material used a nanozyme, but rather a catalytic nano-particle.

Earlier work. The catalytic activity of gold nano-particles (colloidal gold) has already been demonstrated, particularly in oxidative processes involving oxygen and hydrogen peroxide, and the latter include the use of TMB and DAB as the second reactants on an oxidative process with hydrogen peroxide (the so called peroxidase activity). It should be clearly stated what advance on earlier work has been reported here.

Experimental section 2.2. To what pH was the reaction mixture adjusted with 1.0M NaOH?

Experimantal section 2.3. Kinetic measurements. The correct pH of the acetate buffer used with TMB is not given. (Acetate buffering is negligible at pH 0.4). Was it demonstrated that, during the 10 minute incubation with TMB, the rate of reaction was constant? For the experiment(s) with DAB, the situation is quite unclear. It seems that a reaction mixture was incubated for 25 minutes in which time the reaction went to completion, and “thereafter” something was „further probed“. Am I correct in thinking that the reaction was carried out for 25 minutes, that at that time the absorbance was measured, and this was then used to calculate activity? Was the rate constant during those 25 minutes?

The evaluation of results. Figure 2 refers to Michaelis-Menten plots (Figure 2). Those authors did plot a measure of product production (change in optical rotaion) against time, but Figure 2 is of velocity against TMB/DAB concentration. The words „Michaelis.-Menten plot“ are misleading. After many careful corrections to their data, Michaelis and Menten did arrive at an equation with the algebraic form now known as the Henri-Michaelis-menten equation. (Henri had derived the equation earlier, and Michaeis and Menten fully acknowledged the earlier work). Henri in one of his papers had described the results as being possibly hyperbolic, and that is indeed the form of the Henri-Michaelis-Menten equation. It is a rectangular hyperbola. How to deal easily with such kinetics was shown only later by Lineweaver and Burke.

The results presented in the Lineweaver-Burke plots are not adequate. It is not clear if they are results from single experiments, or average results from multiple experiments. If they are the former, they are inadequate, and if the latter then mean results with standard deviations should be plotted. The author should note that the Lineweaver Burke plot leads to a negative intercept on the reciprocal-concentration axis. In the results shown this may occur with TMB but not with DAB.

All of the reported work was carried out at a single concentration of hydrogen peroxide. Was that a “saturating” concentration? As presented, an evaluation of the results is not really possible. (I might point out that in modern work, the use of reciprocal plots of rates derived from primary data are no longer used. The Michaelic constant and Maximum velocity are determined directly from the primary data (product concentration and time) using computer programmes.

Were controls made to determine and correct for rates not caused by the nano-catalyst (in the presence and absence of oxygen)?

Finally, it ought to be made clear in the conclusion how these results confirm or differ from earlier ones made with TMB and DAB

I hope these general comments will help the author review and rewrite his presentation. His English is good, and under the present circumstances I have avoided pointing out small improvements that might be made. I would be happy to do that in a revised presentation.

Eric Barnsley