

## Analytical usefulness of the combined use of Tb<sub>4</sub>O<sub>7</sub> nanoparticles and laccase enzyme for the determination of antioxidants in food samples

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The analytical usefulness of terbium oxide nanoparticles (Tb<sub>4</sub>O<sub>7</sub> NPs) for the determination of phenolic compounds in food samples in combination with the use of laccase enzyme is described for the first time. Laccases are oxidase-phenol enzymes, which are isolated from several types of plants, fungus and microorganisms, the most used variety being *Trametes Versicolor*. Laccase enzyme catalyzes the oxidation of phenolic compounds by reducing the dissolved oxygen present in the medium. This reduction can also be monitored by its action on some chromophores, such as 2,2'-azino-bis-(3-ethylbenzothiazolin-6-sulfonic) acid (ABTS)<sup>1</sup>.

The use of the fluorophore 8-hydroxypyrene-3-sulfonate trisodium (HPTS), which fluorescence decreases in presence of laccase, is now reported. The observed decrease is lower in presence of antioxidant compounds, such as gallic acid, which has been used as model analyte. The areas under the kinetic curves obtained in presence of this antioxidant are directly proportional to its concentration. The presence of Tb<sub>4</sub>O<sub>7</sub> NPs in the reaction medium gives rise to an increased reaction rate, which provides shorter analysis times, and hence, a better sample throughput.

This novel assay has been developed in a 96-well microplate format and it consists of two steps: 1) Pre-incubation of HPTS together with gallic acid standards or samples for 15 min at 37 °C, and 2) the addition of a pre-mixed solution, which contains a mixture of Tb<sub>4</sub>O<sub>7</sub> NPs and laccase. This mixture is placed immediately on the microplate reader and kinetic curves are monitored using filters with nominal excitation and emission wavelengths of 450 and 535 nm, respectively. The method shows a detection limit of 0.14 µM and dynamic range of 0.5 - 12 µM. The precision, expressed as relative standard deviation, has been assayed at two different concentrations, 0.7 and 5 µM, obtaining values between 2.5 and 6.3 %. The method has been applied to the analysis of wine and beer samples, which demonstrates its analytical usefulness.

### References

<sup>1</sup>Branchi, B., Galli, C., Gentili, P., *Org. Biomol. Chem.*, **3** (2005) 2604.