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Critical bioanalytical study of the FRAP assay for assessing total antioxidant capacity of biological samples

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Free oxygen radicals are highly reactive molecules and have been recognized as a major cause of oxidative stress. Molecular oxidative damage, caused by reactive oxygen or nitrogen species (ROS/RNS), have been implicated in the pathophysiology of many diseases including diabetes mellitus, cancer, rheumatoid arthritis as well as cardiovascular, renal, inflammatory, infectious, and neurologic diseases. Biological fluids within the human body have an array of protective antioxidant mechanisms; both for preventing the production of free radicals and for repairing oxidative damage and several assays have been adopted to measure a total antioxidant capacity of biological fluids. However, the measured antioxidant capacity of a sample depends on the method and free radical generator or oxidant used in the measurement. The ferric reducing ability of plasma (FRAP) assay uses an easily reduced oxidant in a redox-linked colorimetric method and is the only assay that measures on the basis of direct electron transfer. A detailed literature survey revealed the use of an excessive stoichiometric concentration of ferric ions in the formation of the FRAP reagent, resulting in extraneous ferric ion in the solution which potentially can cause variability in values of antioxidant capacity of reference standards as well as samples.

The purpose of this study is to critically evaluate bioanalytical aspects of the FRAP assay and establish the accurate stoichiometry for the non-specific half reaction. In this study, the effect of different concentrations of ferric ions on antioxidant capacity has been analyzed. Stoichiometry and kinetics of FRAP reagent formation was evaluated using cyclic voltammetry and the coupling of a post column reaction with HPLC. An overview of significant results will be presented to provide a better understanding of the FRAP assay and will give an insight into problems and issues encountered during the analysis of biological samples.

Biography

Rashida has completed, Master of Science in Biotechnology from Swinburne University of Technology, Victoria, Australia. Currently, she is pursuing PhD in Swinburne University of Technology, in bioanalytical screening and characterization of cardioprotective bioactive compounds from microbial sources, under the supervision of Dr Peter Mahon and Associate Professor Enzo Palombo.

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