Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as reactive oxygen species (ROS). This work reports the assay both lipophilic and hydrophilic antioxidants simultaneously, by making use of their ‘host-guest’ complexes with methyl-β-cyclodextrin (M-β-CD), a cyclic oligosaccharide, in acetonated aqueous medium using the CUPRAC (Cupric Reducing Antioxidant Capacity) method. The oxidation of a polyphenol \( \text{Ar(OH)}_n \) with the CUPRAC reagent can be represented by the reaction equation:

\[
\text{n Cu(Nc)}_2^{2+} + \text{Ar(OH)}_n \leftrightarrow \text{n Cu(Nc)}_2^{+} + \text{Ar(=O)}_n + \text{n H}^+
\]

where \( \text{Cu(Nc)}_2^{2+} \) is the chromophore responsible for the absorbance at 450 nm [1].

M-β-CD was introduced as the water solubility enhancer for lipophilic antioxidants. The trolox equivalent antioxidant capacity (TEAC) values with respect to the modified CUPRAC method – of various lipophilic and hydrophilic antioxidant phenolics were reported for the first time in this work in a unique solvent medium containing a solubility enhancer. Two percent M-β-CD (w/v) in a 90% acetone-\( \text{H}_2\text{O} \) mixture was found to sufficiently solubilize β-carotene, vitamin E, vitamin C, synthetic antioxidants and other phenolic antioxidants. Synthetic mixtures comprised of lipophilic and hydrophilic antioxidants gave the theoretically expected CUPRAC antioxidant capacities, indicating that chemical deviations from Beer’s law were basically absent, and the observed CUPRAC absorbances were additive. This assay was validated through linearity, precision, and accuracy. The validation results demonstrate that the CUPRAC assay is reliable and robust.

The CUPRAC antioxidant capacities of a wide range of polyphenolics and flavonoids were experimentally reported in this work as TEAC values, i.e., the ratio of the molar absorptivity of each compound to that of trolox in the CUPRAC assay, and compared to those found by reference methods, ABTS/HRP-\( \text{H}_2\text{O}_2 \) and FRAP.

Reference