

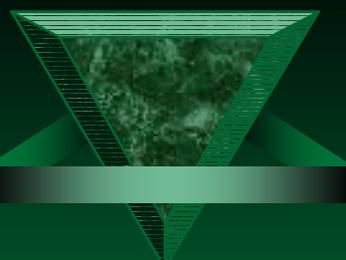
RP-HPLC-ED measurements of the total antioxidant potential: Comparison to the standard photometric method

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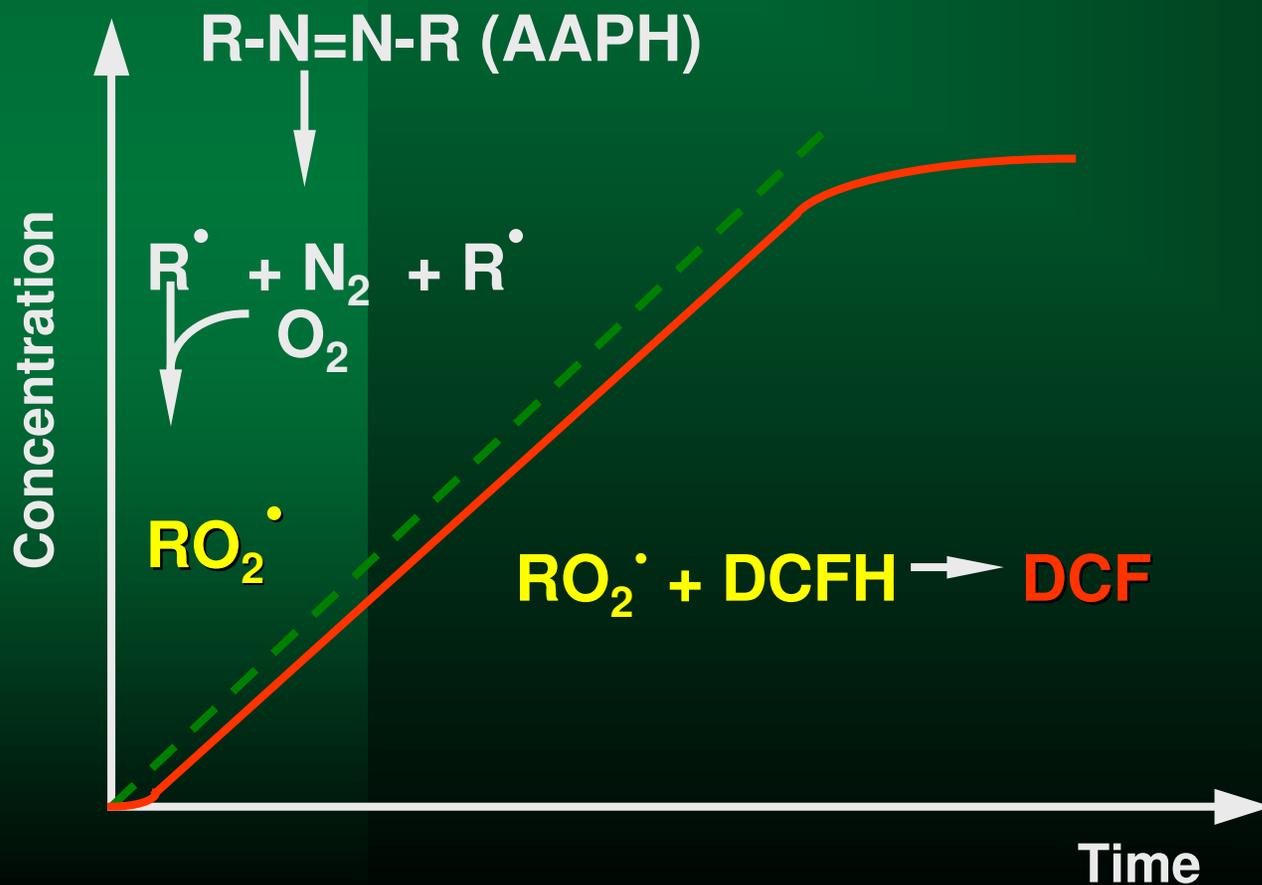
OBJECTIVE

Free radicals adversely modify biologically active molecules as well as whole cells and are implicated in various degenerative diseases and aging. Their mediated process have been implicated in the pathogenesis of several diseases. It is widely believed that these modifications are preventable by exogenous antioxidants. There is a need for a method to assess and compare strength of particular antioxidants in order to select these of the highest potential for further development as drugs. However, it turned out that frequently more information (e.g. synergetic effects) is obtained measuring total antioxidant potential (TAP) of biological samples than concentration of particular antioxidants separately.

Results are presented concerning the application of RP-HPLC to the estimation of TAP after hydroxyl radicals generation in the Fenton reaction and their spin trapping with hydroxybenzoate. Samples were monitored using direct current amperometric detector with the glassy carbon electrode, worked at the potential 0.8 V vs Ag/AgCl electrode.

PHOTOMETRY

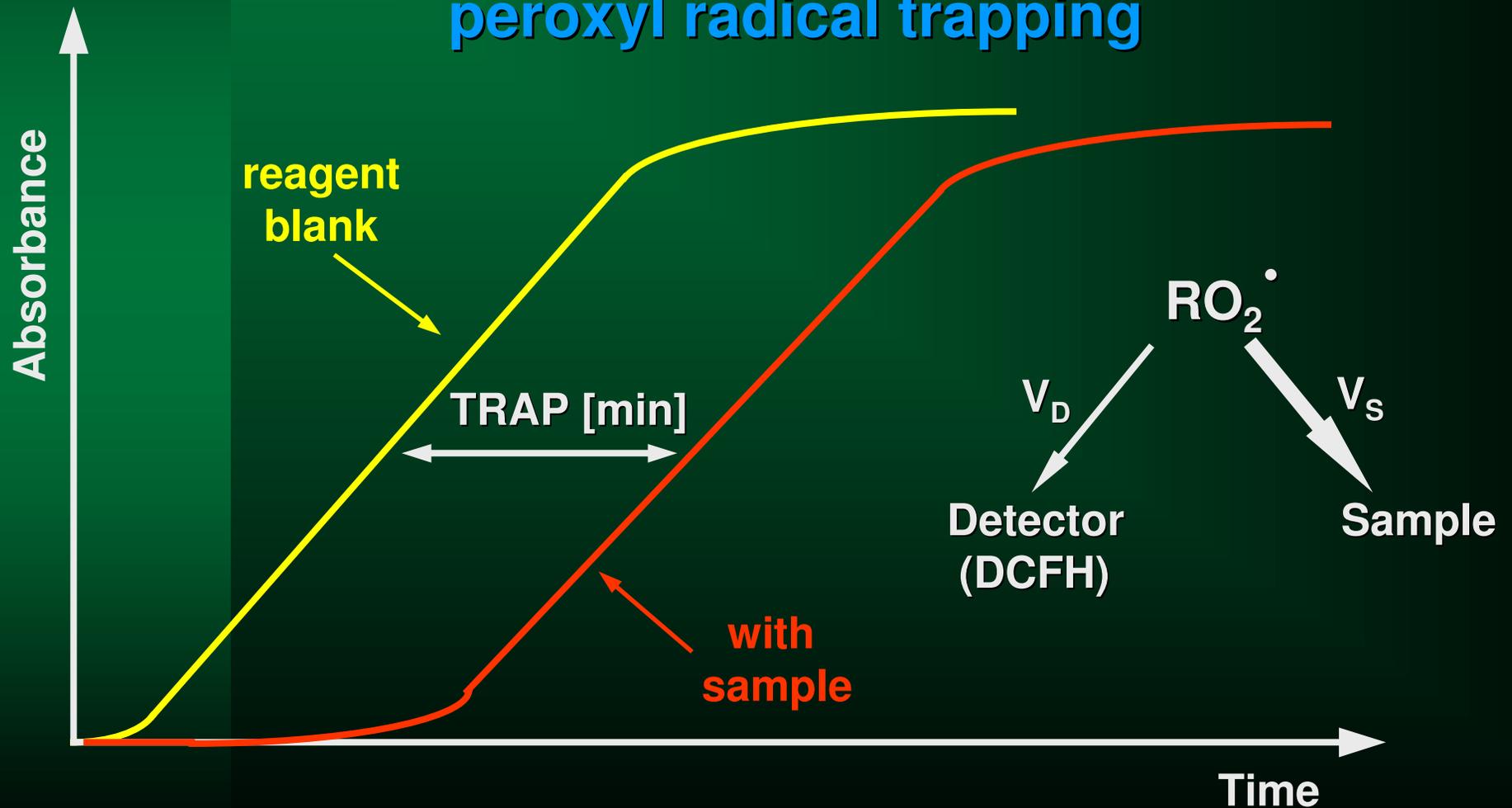
Generation and detection of peroxy radicals

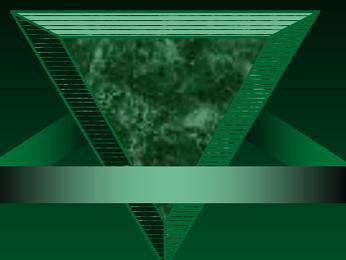


Photometric measurements are based on the modified Valkonen and Kuusi method [25]. Peroxyl radicals were obtained from thermal decomposition of AAPH (final concentration – 56 mM). In the first step carbon radicals are formed in pairs, which react rapidly with oxygen molecules to give peroxy radicals. Their concentration was monitored photometrically, at 504 nm, measuring the conversion of DCFH·DA to dichlorofluorescein (DCF). The reaction was performed in 50 mM PBS solution at 26 °C. DCFH·DA was dissolved firstly in DMSO followed by the same amount of water to obtain a final concentration of 14 mM. Results are described as the delay time (measured at half time of the reaction) of competition kinetics during which antioxidant is consumed. This parameter measures the total antioxidant reactivity and is defined as the sum over all antioxidants present in the sample, of the product of reaction rate constant and concentration. The measurements of the antioxidant capacity were repeated 3 times for each sample and the results were averaged and expressed relative to the average result for the control samples containing no sample. Results were recalculated to TEAC [mM] (Trolox Equivalent Antioxidant Capacity).

PHOTOMETRY

Kinetics of absorbance during peroxy radical trapping





HPLC

Generation and detection of hydroxyl radicals



ADP

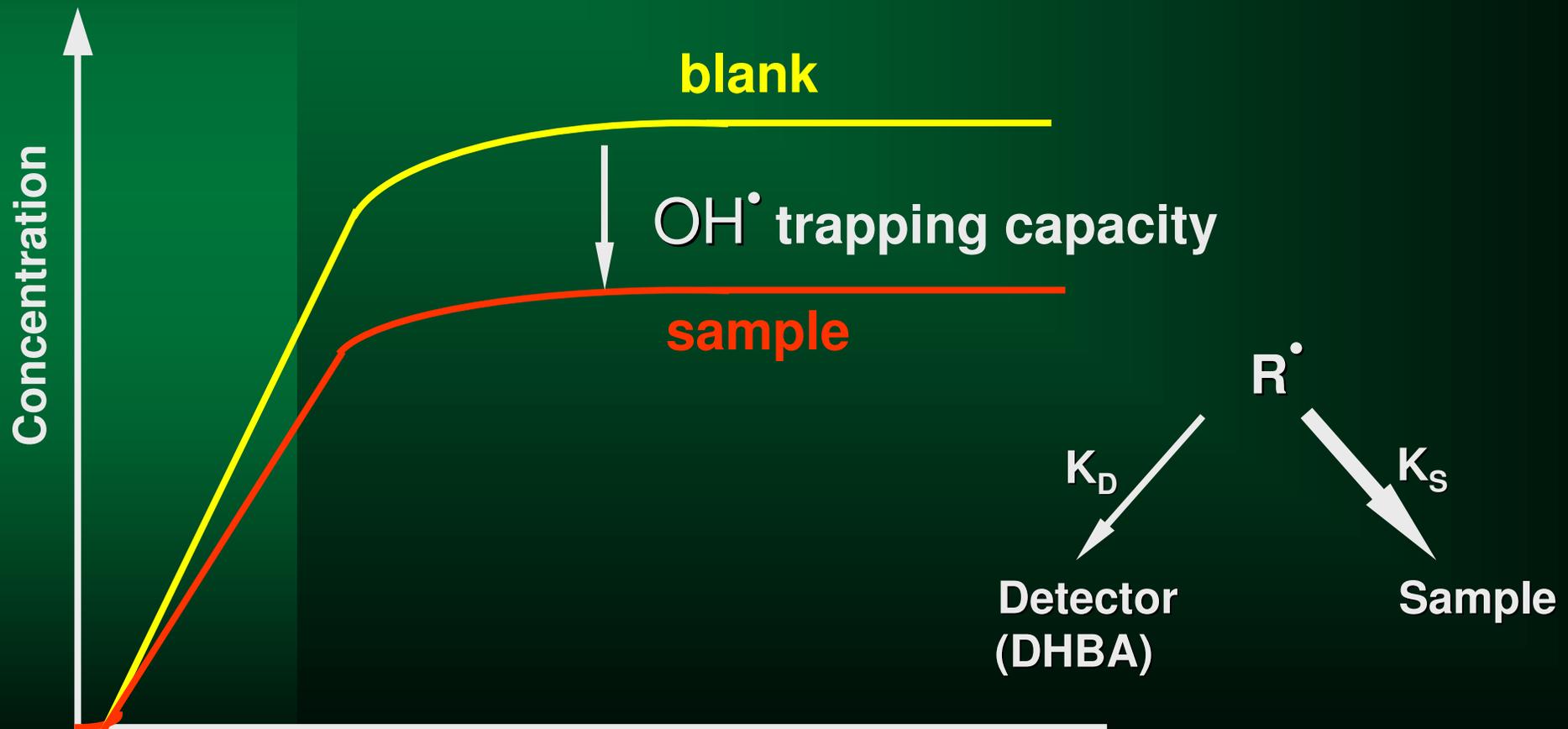


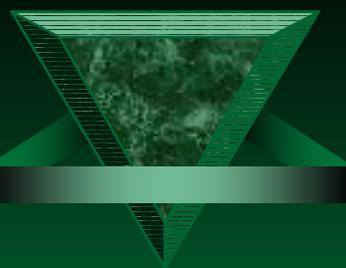
Chromatographic
assay

Hydroxyl radicals were generated through Fenton reaction [11] by 1 min incubation of 0.5 mmol L⁻¹ Fe²⁺, 2 mmol L⁻¹ ADP, and 2 mmol L⁻¹ H₂O₂ in 50 mmol L⁻¹ phosphate buffer (pH 7.4) in the presence of 1 mmol L⁻¹ *p*-hydroxybenzoic acid and analyzed sample at 37 °C. The reaction was stopped by 2 mmol L⁻¹ DMSO and 0,1 g/mL Desferal, and the reaction mixture was immediately analyzed by HPLC.

HPLC

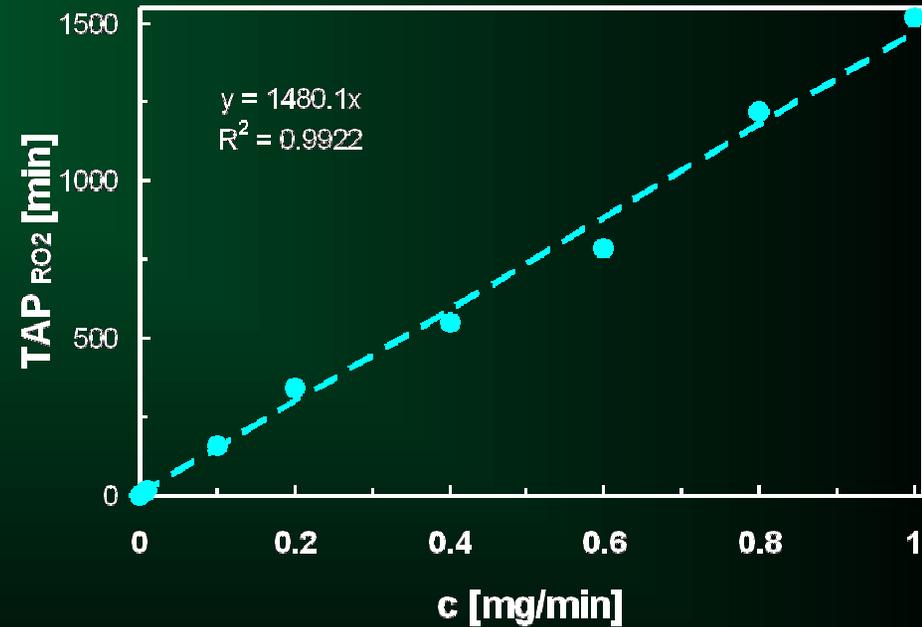
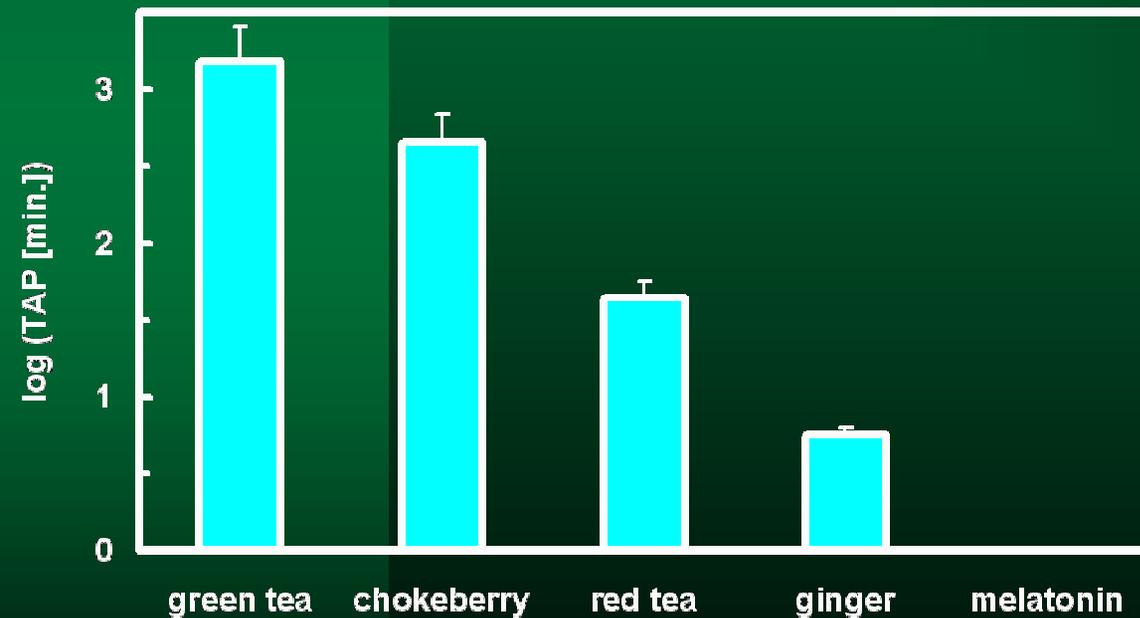
3,4-DHBA concentrations as the measure of OH^\bullet free radical trapping





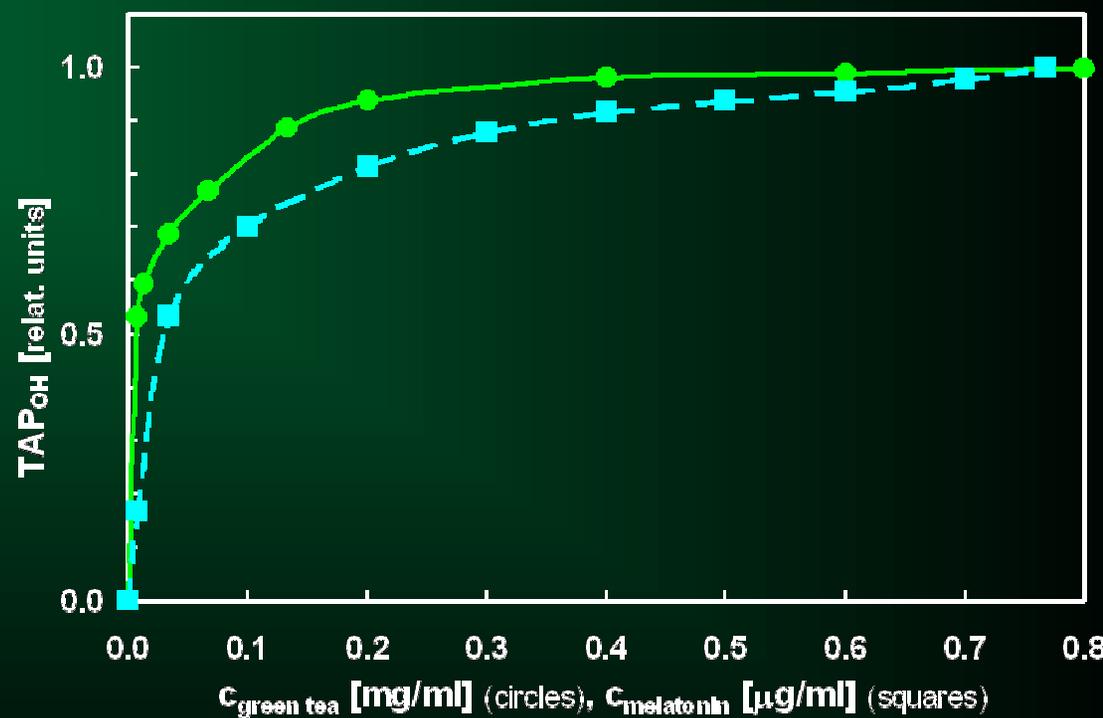
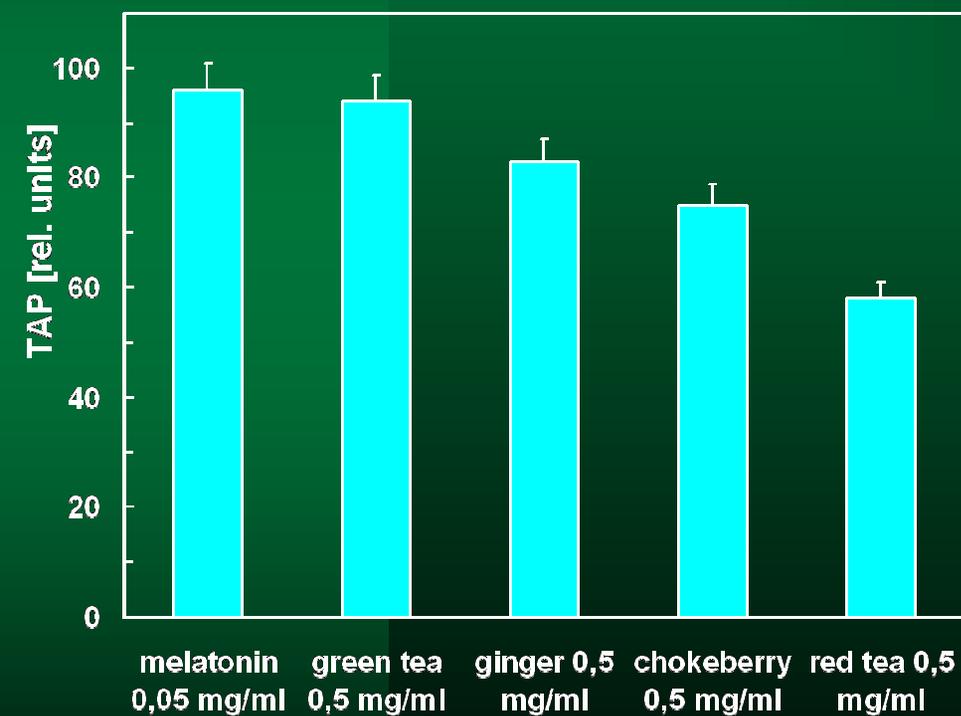
PHOTOMETRY

TAP of 1 mg/ml herbal extracts

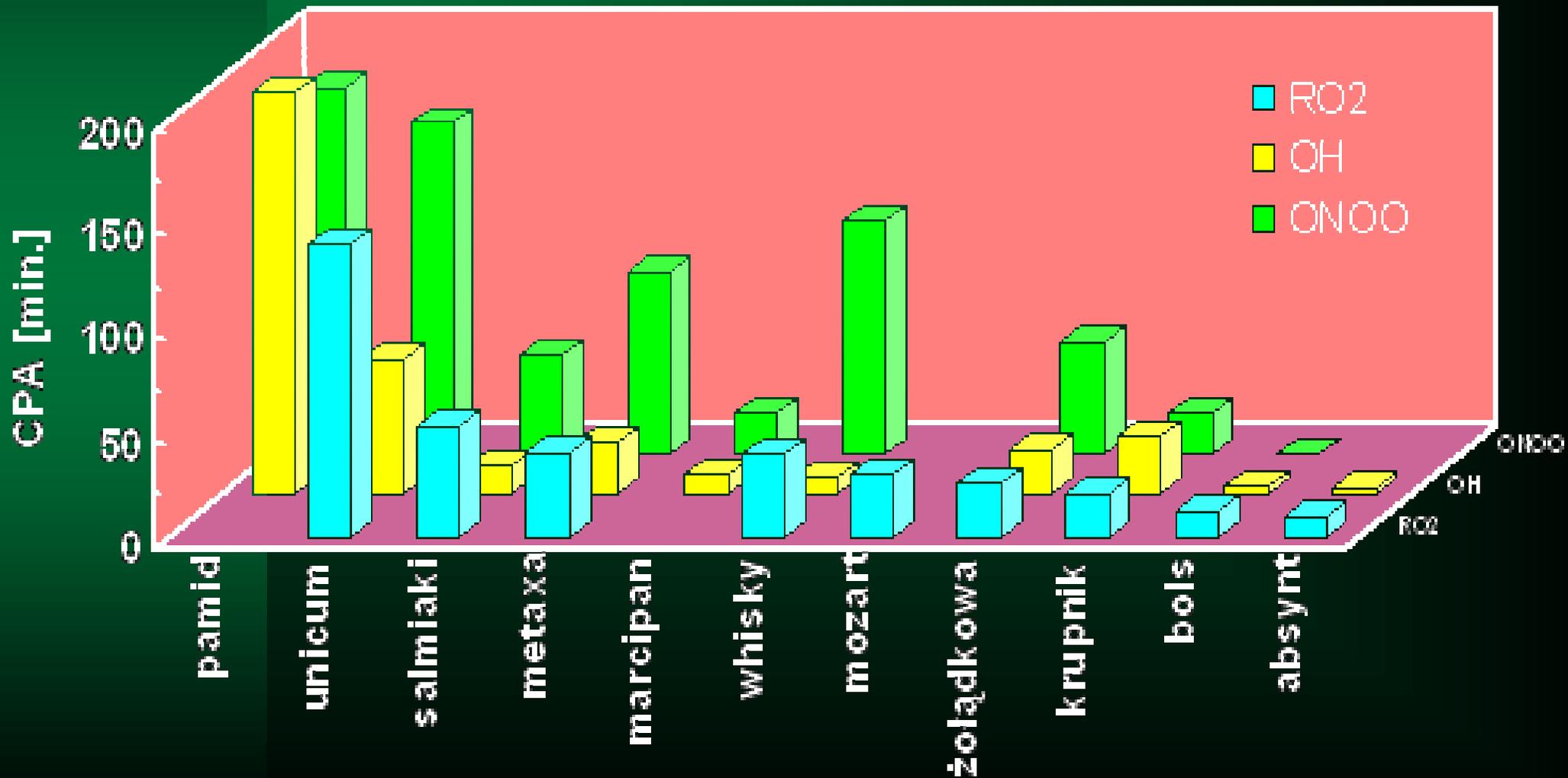


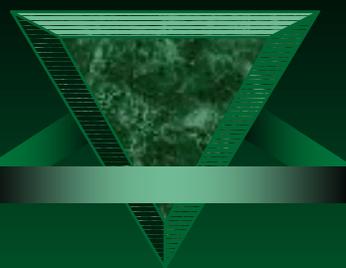
HPLC

TAP of 1 mg/ml herbal extracts



Wine-therapy





CONCLUSIONS

It turned out that HPLC can be used to the measurements of TAP giving additional information.

The method was applied to the determination of TAP of some types of compounds (biogenic polyamines, catecholamines and polyphenols), herbs and blood serum. The results were compared with those obtained using photometric TAP method based on peroxy radicals generation by thermal decomposition of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) in the presence of assayed sample and detection with 6-carboxy-2',7'-di-chloro-dihydrofluorescein diacetate. It turned out that relative value of the antioxidant potential strongly depended on the generated radical.