# Blood serum demonstrates antioxidative mechanism: a Magnetic Resonance Relaxation Studies

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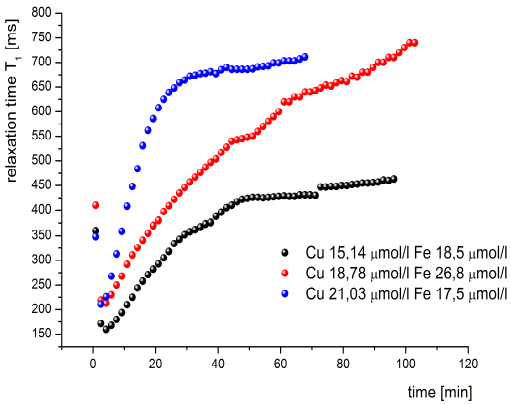
**Introduction:** Reactive oxygen species **(ROS)** were indicated as a factor accelerating ageing process. There are produce in a number of biological processes. Cytotoxic action of ROS includes protein, lipid and DNA structure damage and can be primer to many human diseases, such as cancer, Parkinson's, Alzheimer's, and many others. ROS have paramagnetic properties, they significantly shorten NMR relaxation times. Hydroxyl free radicals can be produced from hydrogen peroxide in a Fenton type reaction. We present some NMR relaxation results which can be used to very important studies of oxidative processes. So far only EPR and CIDNP methods have been used to observe the action of free radicals in biological systems. Here, we compared the effects of adding hydrogen peroxide to human blood serum and selected protein solutions on relaxation times to evaluate their antioxidative effects.

**Methods:** Hydrogen peroxide was added in-vitro in relation 1:10 to human and rabitt blood serum, lyophilized bovine serum albumin, egg white albumin and $Fe^{2+}$ aqueous solutions. Fe and Cu concentrations in blood serum samples were estimated using spectrophotometry methods. Proton NMR measurements of relaxation times were performed using a 60 MHz Minispec Bruker spectrometer. Spin-lattice relaxation time $T_1$ was measured by the Inversion Recovery (IR) sequence, spin-spin $T_2$ by CPMG method. Temperature of samples was stabilized and measured with accuracy ±1°, most of measurements were done at room temperature (+23°C). Finally, experiments were repeated with various concentrations of vitamin-C or glutathione added to blood serum.

**Results:** When $H_2O_2$ is added to blood serum, which naturally containing Fe and Cu ions, one of well known an oxidation reaction, named Fenton reaction, take place and as a results the free radicals (ROS) are produced. The presence of ROS influences relaxation processes and changes the relaxation times of biological samples. Figure 1. illustrate the change in relaxation times as a function of time in blood serum. Please note increase in $T_1$ of blood serum after initial drop due to addition of hydrogen peroxide. This process was not observed in some ingredients of blood (hemoglobin, albumin and iron salt) which were separately performed. Interestingly, after addition of vitamin C and/or glutathione, the initial drop in $T_1$ is smaller than in blood serum, suggesting that these substances enhance the antioxidative properties of blood serum (figure 2).

**Conclusions:** ROS created from hydrogen peroxide in Fenton reaction significantly shorten the relaxation times. However, increase in relaxation time $T_1$ was observed in blood serum, but not in some ingredients of blood serum. The mechanism likely reflects removal/deactivation of ROS by some components of blood serum. More importantly, this antioxidative mechanism is enhanced by addition of known antioxidants: vitamin-C and glutathione. Antioxidants concentration dependence of relaxation behavior show that presence of these media in solution restrain progress of oxidation. Our results indicate that measurements of relaxation times may be used for the studies of oxidative processes in biological liquids, and in the future, be helpful in investigation of some human diseases, specially with free radical background.



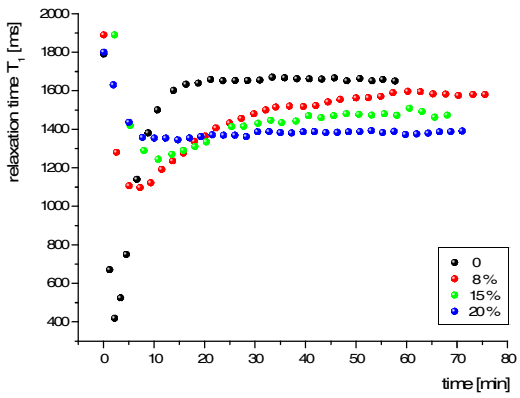




Figure 1: $T_1$ relaxation of blood serum after addition $H_2O_2$ (3%)
Figure 2: $T_1$ relaxation of blood serum after addition vitamin C in different concentrations