

UV-VIS Technique for Comparison of Oxidative Stress Markers in Diabetes Types 1 versus Type 2

M. Valcheva-Traykova¹, G. Bogdanov², G. Bocheva²

¹Department of Medical Physics and Biophysics, Medical Faculty, Medical University, Sofia, Bulgaria

²Department of Pharmacology and Toxicology, Medical Faculty, Medical University, Sofia, Bulgaria

Abstract. Measurements of Oxidative Stress (OS) markers using UV-VIS spectrophotometers are widely used in assessment of the OS levels in tissues of model animals and humans. In the present investigation, the accumulation of free radicals in the rat blood serum, in two models of diabetes mellitus was estimated, using UV-VIS spectrophotometer. Diabetes Mellitus (DM) was provoked either by subcutaneous injection with Streptozotocin (100 mg/kg) 3 days after birth (group “Str”), or via fat-rich diet (group “Palm Oil”). The third “Control” group was left untreated. The model animals were kept in agreement with the General regulations for treatment of experimental animals. After decapitation under anesthesia (Uretan, 2 mg/100 g BW), the blood was collected, blood serum was extracted and used for analysis. The formation of MTT- formazan ($\lambda = 576$ nm) and the accumulation of uric acid ($\lambda = 293$ nm) for 5 minutes, were used as markers for the overall free radicals formation, and accumulation of Reactive Oxygen Species (ROS), respectively.

UV-VIS measurements were performed using Shimadzu 1600 UV-VIS spectrophotometer equipped with software, and quartz cuvette, in 50 mM K,Na-PBS (pH 7.45). One ml of the cuvette contained blood serum corresponding to 1 mg. proteins and 0.01 ml 3mM Xanthine dissolved in PBS to 1 ml; the MTT – formazan formation was monitored in the presence of 0.05 ml MTT dissolved in H₂O.

More free radicals and higher activity of Xanthine oxidase were found in the blood serum of Streptozotocin-induced than in high dietary fat – induced diabetic rats. It was concluded, that UV-VIS technique may be used to estimate the relative differences within OS levels in the blood serum of rats suffering from diabetes mellitus types 1 and 2.