ABSTRACT

The main goal of this dissertation was to develop original analytical systems based on the high-performance liquid chromatography (HPLC) technique and emission spectroscopy (fluorescence, chemiluminescence) for the determination and testing of the properties of selected water-soluble vitamins in whole blood and in dialysis effluents.

The developed analytical systems were compared in clinical assays with the existing solutions in terms of their applicability in modern biomedical analytics. Their usefulness was determined using both classical approaches of statistical analysis and modern chemometric tools. The developed methods were employed in the analysis of whole blood samples of 50 regularly hemodialysed patients and 41 patients treated with peritoneal dialysis. In addition to this practical aspect, the physicochemical properties of the investigated systems were also determined, including the kinetics of formation of fluorescent target analytes, as well as fluorescence lifetimes of such derivatives, that are – thiochromes.

As part of the research, the concentration of the active form of vitamin B_1, i.e., thiamine diphosphate (ThDP) in whole blood was determined at the average level of 50.6 ± 47.3 ng/ml before hemodialysis and 24.1 ± 23.5 ng/ml in samples taken immediately after this procedure. A significantly differentiated loss of ThDP concentration was observed among patients, ranging from a few percent to values close to 100% in some cases. The comparison of the results obtained with the use of the developed method with the validated commercial test confirmed its high quality, repeatability of the obtained results, and thus – usefulness in clinical trials. The application of principal component analysis (PCA) revealed the dependence of the level of the assayed vitamin on the type of dialyzer, dialysis membrane used, diuresis, creatinine and hemoglobin concentration, as well as the number of red blood cells.

Determination of vitamin B_1 and B_6 concentration was performed in a matrix not previously tested in this respect, that is in dialysis effluents. The samples were collected during the peritoneal equilibrium test (PET) at the 2-nd and 4-th hours of the test, respectively, and after the 24-hour replacement. It turned out, that peritoneal dialysis procedures, like hemodialysis, also predispose to loss of vitamins B_1 and B_6 from the body of patients, but in this case the one is significantly lower than in the case of HD. Despite very low concentrations of analytes in the matrix, it was possible to determine

the concentrations in peritoneal dialysates, which ranged from 0.43–6.88 ng/ml for thiamine monophosphate, 0.45–50.24 ng/ml for thiamine diphosphate, 0.08–2.61 ng/ml for pyridoxine, 0.09–17.26 ng/ml for pyridoxal and 0.08–3.64 ng/ml for pyridoxamine. The determined vitamin content was lower than the recommended daily intake and did not exceed the excreted amount with urine by healthy people. The washout level increased with the duration of the PET treatment and in many cases was nearly two-fold higher for the samples taken after the 24-hour exchange as compared to the samples collected at the 2-nd hour of this test. However, the applied statistical methods did not reveal any significant relationships between the concentrations of subsequent analytes with other clinical parameters describing the studied group of patients.

An additional aim of this dissertation was to determine the possibility of using the chemically induced luminescence process to assess the antioxidant properties of biologically active compounds and dietary supplements, including water-soluble vitamin C (ascorbic acid). In this original approach, specially synthesized acridinium salts, including 10-methyl-9-(phenoxycarbonyl)-acridine triflate, were employed as chemiluminogenic indicator. The proposed method is based on the measurement of changes in the emission parameters of the last compound (such as the kinetics of emission decay) from an alkaline hydrogen peroxide solution in the presence of antioxidants, in comparison to the emission observed without such additives. Due to the beneficial, flash kinetics of emission of the acridinium indicator, only a negligible influence of the color or turbidity of the studied sample on the analytical performance of the method was observed. The latter observations make it more universal and sensitive than classical approaches based on the measurement of the efficiency of luminol chemiluminescence.