# Multivariate data analysis in biomedical spectroscopy: cholinesterase activity determination

*Nechaeva N.L., Kurochkin I.N.*
*Chemical enzymology department, Faculty of Chemistry,*
*Lomonosov Moscow State University*

Surface-enhanced Raman spectroscopy is a modern powerful tool for the analysis of complex biological samples. The use of silver pastes is one of the most common SERS applications on the practice. In the current work silver screen-printed SERS-substrates were used. This developed technique is inexpensive, easy-to-use and do not required special equipment. The application of the SERS-effect is expedient in the case of the analysis of complex biological objects, for example human blood enzymes. Acetylcholinesterase and butyrylcholinesterase catalyze the hydrolysis of many physiologically important compounds such as neurotransmitter acetylcholine, caffeine, and cocaine. The activity of these enzymes in the blood may be lowered due to poisoning by organophosphorus compounds, pesticides, and also due to various diseases. Therefore it is very important to measure cholinesterase activity in blood with high accuracy to assess toxicity and for diagnosis of diseases at early stages. The best known method for determining the activity of AChE and BChE is to measure butyrylthiocholine (BTCh) and thiocholine (TCh) – substrate and product of the enzymatic reaction, respectively. We used a silver print-screen surface as the SERS-active structure for the analysis of BTCh and TCh, with subsequent determination of enzyme activity.

Raman spectra are a large amount of data, which includes information about the composition of the substance, as well as various noises. To assess the contribution of each peak in the spectrum and make quantitative measurements of the spectrum is a very difficult task due to the sheer amount of information obtained from the spectra. For statistically significant allocation of contributions and for accurate quantitative analysis we used multiparametric statistical methods. In our work we used the principal component analysis (PCA) method for the separation of spectra of substrate and product of the enzymatic reaction, and the method of PLS to construct a calibration curve and further define the unknown samples. Ellman's assay was used as reference method to validate enzymatic activity values, measured by Raman spectroscopy.