Raman spectroscopy is an optical vibrational spectroscopic technique suited for a study of biomolecules and their interactions. In contrast to classical Raman spectroscopy, Drop-Coating Deposition Raman spectroscopy (DCDR) enables due to a preconcentration of the sample dried on a special hydrophobic surface to acquire a high-quality spectrum even in case of low concentrated samples. In collaboration with Department of Macromolecular Physics of the Faculty of Mathematics and Physics at Charles University, the three special surfaces – substrates – with different wettability (hydrophobicity) and roughness on the basis of silver nanoparticles have been prepared. Solutions of methylene blue and glutamic acid at different concentrations and pH were dropped on these substrates. Nanoparticles size was determined. It was shown, that with increasing number of sputtered nanoparticles, the roughness of the substrates increased also. More roughened substrates show a higher static contact angle. The dynamics of droplet drying and the preconcentration of substances in the dried deposit were monitored. The preconcentration was assessed from the first subspectrum obtained from a factor analysis performed on series of measured Raman spectra from dried deposits. There was none among the substrates on which there would generally be a better preconcentration, on the contrary, this preconcentration was found to be very similar for all substrates. The roughness of the substrate did not influence the preconcentration of the studied substances and the intensity of the signal was very similar for all studied substrates. Different trends for different pH values and different concentration values were not observed.