# Optimization of selected click reactions and their applicability in On-Line labeling in CZE

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Capillary zone electrophoresis (CZE) is not only an efficient and reliable separation technique but it is often advantageously used for determination of physico-chemical parameters of various compounds [1,2] or characterization of kinetics of chemical reactions [3,4]. These data offer deep understanding of desired separation systems or chemical reactions. Simultaneously, CZE is very well described theoretically and there are several simulation programs available, which offer deep insight and understanding of separation process, and allow optimization of separation conditions. One of the powerful simulation programs is Simul 5, which offers broad range of features to simulate special modes of electrophoresis, i.e. complexation, kinetic or temperature mode.

Cu free click chemistry finds broad applications in the monitoring of biologically important processes [5]. However it still fights various limitations as the poor solubility of reactants in aqueous solvents or low reaction rates. In this study we present an electrophoretic approach to characterize the kinetics of the azide-alkyne Huisgen cycloadditon. We introduce a fast and reliable CZE method to determine reaction kinetics of Cu free click reactions based on the established theoretical model. The method and its benefits were verified theoretically using Simul 5 Kinetic. We demonstrate the benefits of the method on tuning of reaction conditions in terms of pH of reaction buffer and presence of buffer additives. We were able to show that varying the pH of the reaction buffer can result in significant increase in the reaction rate of the Cu-free click reaction, by influencing the charge state of the reactants. This effect was confirmed by DFT analysis. Further we propose the utilization of micelles to enhance the reaction kinetics as well as to improve the solubility of reactants.

In conclusion we were able to enhance the rate of reaction up to the factor 100, and thus select the optimal reaction conditions. We also propose the method to perform the On-line Cu free click cycloaddition followed by effective separation of reactants and products within the single run. This method can be easily used for On-line Cu free click labeling of biologically important compounds and the consequent analysis of the reaction mixture.

References:
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