

INVESTIGATION OF ANTIBODIES AGGREGATION AND STABILITY OF AGGREGATES

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A response of the immune system for the presence of a foreign, harmful substance – antigen- is the production of antibodies (Ab), also called immunoglobulins. In addition to their main function- to eliminate antigens- antibodies are also used as probes in diagnostics, in target therapy of a wide variety of diseases or in research as a part of analytical methods. When they are produced industrially, they form a part of the cultivation media in addition to a variety of other compounds such as proteins, DNA, etc. As antibodies are used as therapeutics, their high purity is necessary. In practice, Ab are purified by several chromatographic steps. The number of chromatographic purification steps could be reduced by the use of columns with multimodal adsorbents, which provide more than one mechanism of binding impurities. The aim of our work was to prepare a model mixture for subsequent experiments in which mixed-mode chromatographic purification of a monomer from aggregated forms and impurities takes place. In the first part of our research, we investigated different conditions in which the aggregation of the original monomer antibody form occurs. Experiments with decreasing pH from 5 to 3.5 and subsequent addition of acid-hydroxide were performed. These methods do not cause significant changes in aggregate amounts. In the next step the freezing-thawing method was applied. The samples were frozen at -80°C for 20 minutes and thawed at 25°C for 15 minutes in 5 cycles. In addition, in this experiment, we did not observe a change in aggregates concentration. The third stressing factor tested was temperature. With increasing temperature, an increase of aggregate amount was observed. The second part of our work was devoted to testing the stability of the aggregates in the presence of different salts. Sodium thiocyanate slightly reduces the level of aggregates in contrast to sodium sulfate, which supported the aggregation.

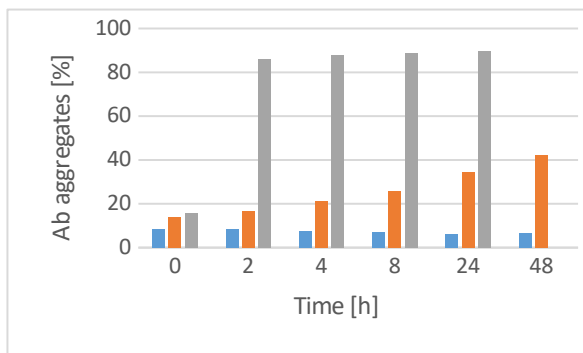


Figure 1: Aggregation of Ab at different incubation temperatures: ■ 40 °C, ■ 60 °C, ■ 80 °C

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