

Software for Automatic Buffer Production



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In order to efficiently determine the optimal conditions for protein purification, methods for downstream processing of biopharmaceuticals are now developed with Design of Experiments and automated systems. Previously, the manual preparation of numerous buffers with different ionic strengths and pH values was a time- and labour-intensive step. We have therefore developed software that allows defined buffers to be produced in a few minutes using a liquid handling system.

Screening Applications

In downstream processing, systems with a 96-well format are used, which enables efficient screening of the conditions for protein purification. For this purpose, 96-well plates with chromatography material (e.g. Atoll columns, Atoll; PreDicator Plates, GE Healthcare) or with membrane adsorbers (e.g. Vivawell, Sartorius Stedim Biotech) are commercially available. The screening can be carried out automatically with liquid handling systems. For purification, the appropriate composition of the loading, washing and elution buffers needs to be determined; in all buffers the particular ionic strength and the exact pH play an essential role.

BufferCalc96 Software

To the best of our knowledge there was previously no system on the market that provides automated buffer preparation with defined pH values and ionic strengths for high-throughput process development. We have therefore developed such a system in the Centre for Biochemistry. The core of the system is the BufferCalc96 software, which calculates the volumes of the stock solutions used. For this purpose, the software uses an iterative procedure, based on the Debye-Hückel theory, to calculate the composition of a buffer with a defined ionic strength and pH value.

In the BufferCalc96 software, the experimenter defines the microplate layout, the buffer system and the input parameters such as buffer concentration, pH value, ionic strength and temperature, as well as the four stock solutions, which consist of the acid and basic components of the buffer, a salt solution, and water.

The software calculates the exact buffer composition and transfers the volume to be pipetted to the software of the liquid handling system, which prepares the buffer from the stock solutions provided. During the development of the software, the pH values and ionic strengths of the buffer prepared by the liquid handling system were verified. The software provided very good matches with the reference values (Fig. 1) for all the buffers tested (phosphate, Tris-HCl, acetate, formate, MES and CAPS).

Practical Implementation

The BufferCalc96 software was tested for screening the purification conditions for a monoclonal antibody with a membrane adsorber 96-well plate. The software enabled the optimum conditions to be calculated (with double determination) from 48 different buffer conditions which covered four ionic strengths and 12 pH values. Figure 2 shows the amount of target protein in the eluate as a function of pH and ionic strength.

Conclusion

The software developed by the ZHAW makes it possible to produce 96 different buffers with defined ionic strengths and pH values in just

9 minutes. A licence to use the software has been granted to the liquid handling system manufacturer Tecan and the software has been commercially available under the name Buffer Creation Wizard since October 2011.

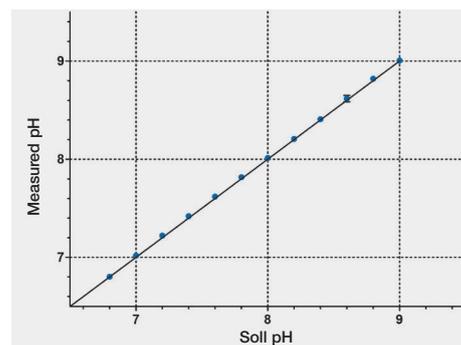


Fig. 1: Validation of software by the Tris-HCl buffer produced using the BufferCalc96 software. The pH values measured closely match the target pH values ($R = 0.9997$).

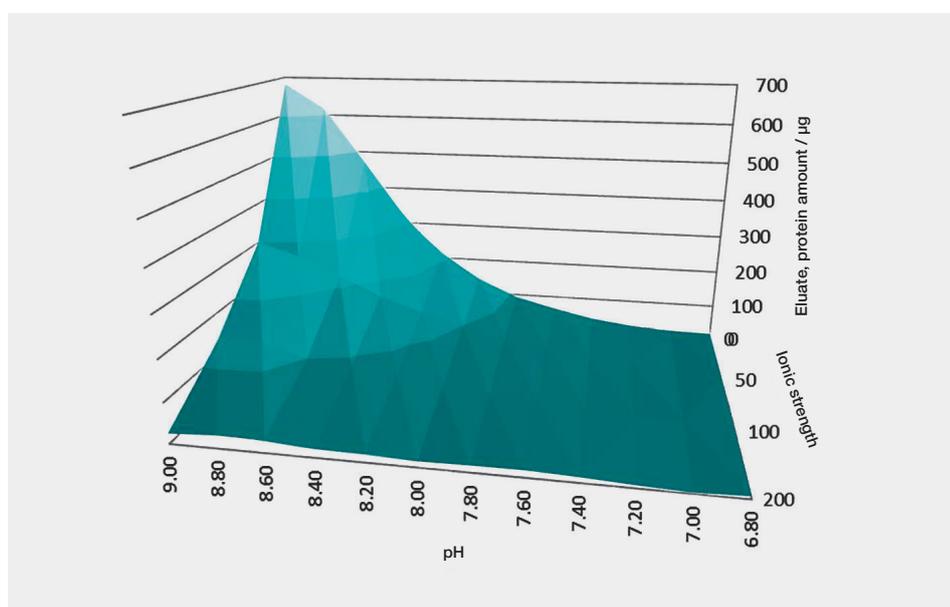


Fig. 2: Calculation of the optimal conditions for the purification of a monoclonal antibody with a membrane adsorber 96-well plate.