INTRODUCTION

Separation of proteins can be achieved with high resolution, reproducibility and sensitivity by cIEF. These include applications for downstream analysis, purification and screening. The method is used to separate proteins based on their charge differences, allowing for the identification and quantification of different isoforms. This technology is particularly useful for the analysis of complex mixtures, such as proteomes, where the high sensitivity and specificity of cIEF can be advantageous.

EXPERIMENTAL SET-UP

For the separation of proteins by cIEF, the following equipment is typically used:

- Capillary electrophoresis apparatus
- UV detector
- Injection system
- Data acquisition system

Separations are performed using neutral capillaries, which are advantageous because they minimize electrophoretic mobilities and improve resolution. The separation temperature is controlled to optimize the separation efficiency and reduce the detection time.

VARIABLES IN cIEF

- pH gradient: The pH gradient is critical for the separation of proteins by cIEF. The pH gradient is typically generated using carrier ampholytes, which are composed of mixtures of pH-independent and pH-dependent ampholytes.
- Concentration of ampholytes: The concentration of ampholytes can affect the separation efficiency and resolution. Higher concentrations can result in improved resolution, but may also increase the detection time.
- Sample injection: The sample injection method can affect the separation efficiency and resolution. A proper injection method is crucial to ensure efficient sample loading.
- Temperature: Separation temperature is an important parameter that can affect the separation efficiency and resolution. A higher temperature can result in faster separation times, but may also affect the stability of the samples.

CONCENTRATION OF cIEF REAGENTS

The concentration of ampholytes in the sample can affect the separation efficiency and resolution. Higher concentrations can result in improved resolution, but may also increase the detection time.

FOCUSING TIME

The focusing time is the time it takes for the pH gradient to develop in the capillary. The focusing time is typically determined by the pH gradient and the concentration of ampholytes. A longer focusing time can result in improved resolution, but may also increase the detection time.

CONCENTRATION OF cIEF MARKERS

The concentration of ampholytes in the sample can affect the separation efficiency and resolution. Higher concentrations can result in improved resolution, but may also increase the detection time.

Peptide pI Markers

The use of synthetic peptides as markers increases the precision and accuracy of the pI determination since they are not complicated with post-translational modifications as in native proteins. The pI value of a peptide can be determined with greater accuracy and reliability, which is particularly important for the analysis of complex mixtures.

Peptide pK Markers

The pK value of a peptide can be determined with greater accuracy and reliability, which is particularly important for the analysis of complex mixtures.

Separation Temperature

Separation temperature can have a significant impact on the cIEF resolution of IgG1. Increasing the separation temperature will decrease the detection time (Figure 11). However, pI analysis is better performed at temperatures above 30 °C. Overall, the use of a higher separation temperature can result in a decrease in the detection time, but it may also affect the stability and charge of the proteins.

RESULTS OF AN INTERMEDIATE PRECISION STUDY

An intermediate precision study was carried out to determine the variability of the cIEF separation under different conditions. Experimental conditions were described in Figure 11 at a separation temperature of 30 °C. The results show that the intermediate precision of the cIEF separation is sufficient for the analysis of complex mixtures.

CONCLUSIONS

Separation of proteins by cIEF can be achieved with high resolution, reproducibility and sensitivity by adjusting the variables. These variables include focusing time, concentration of ampholytes, temperature, and the pH gradient. Understanding the effect of each variable on the cIEF separation is critical when developing a separation method.

Note: For Research Use Only. Not for use in diagnostic procedures.