Separation and Detection of Protein Isoforms Using OFFGEL, Lab-on-Chip Electrophoresis and Mass Spectrometry
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Background
Separation and analysis of charge heterogeneity in recombinant protein or monoclonal antibody (mAb) production is a prime quality control step in the biopharmaceutical industry. This step is often carried out by a combination of separation techniques followed by mass spectrometric detection. Two dimensional gel electrophoresis (2D-GE) is put to use in instances where a tedious procedure, there we present a combination of two easy methods that separate proteins in analogy to 2D-GE according to pl and molecular weight (kDa) with high reproducibility for the analysis of mAb's and recombinant proteins followed by mass spectrometry (MS) analysis. For the 1st dimension, OFFGEL electrophoresis was used. This method takes advantage of the impressive resolving power of immobilized pH gradient (IPG) gels but in contrast to conventional IEF delivers sample in liquid-phase. Fractions with charged isoforms in solution can directly be analyzed by MS.

Results and Discussion
Antibody isoform separation using OFFGEL fractionation and Bioanalyzer high sensitivity protein detection

Hydrophilic capillary gel electrophoresis for protein analysis

Chip HPLEC-Mass spectrometry

Conclusions

References

2. Wenz C and Hörth P., Efficient protein fractionation and identification with the Agilent 5100 OFFGEL fractionator. Agilent Application Note publication number: 5989-8514EN