CAPILLARY ELECTROPHORESIS WITH ON - LINE FTIR DETECTION FOR THE SEPARATION OF PROTEINS

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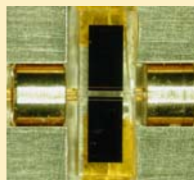
INTRODUCTION

Here we present the on-line hyphenation between capillary electrophoresis and FT - IR [1] applied to the study of the secondary structure of proteins. FT - IR offers the advantage of real - time, non - destructive and molecule - specific detection as compared to UV, LIF or MS. Therefore FT - IR can be employed as a detection method complementary to the above mentioned techniques. The position of the C = O stretch vibration (the so-called amide I band) can be used as an indicator for the secondary structure of a protein. In helical structures the vibration is centered around 1650 cm^{-1}, while in pleated structures it lies around 1635 cm^{-1}.

The main problem when using FT - IR in aqueous solution was overcome by using heavy water (D_2O) where the solvent absorption bands are shifted away from the amide I towards lower wavenumbers. The detection was performed in a micro-machined IR transparent flow - cell [1] and checked using a UV detector to ensure that the proteins were separated as detected in the IR spectrum. The proteins separated were myoglobin, α - lactoglobulin and β - lactalbumin (consisting of two isoforms).

THE FLOW CELL

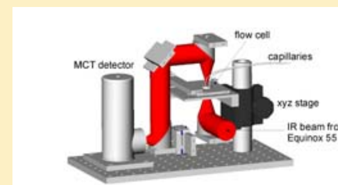
The flow cell is a sandwich of CaF2 plates and epoxy polymer photoresist forming a flow channel of 150 µm width, 15 µm height and 2 mm length.



Microscopic view of the cell with connected capillaries

View of the supporting block with cell and capillary inserted

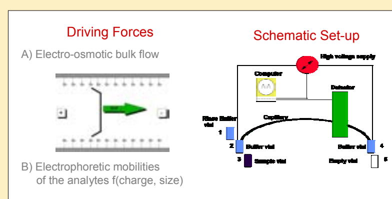
OPTICAL SETUP



Home made high-throughput beam condenser
Focus Area ~ 1 mm diameter

The IR beam from the spectrometer is focused on the flow channel of the CE – FT - IR cell by means of a parabolic mirror and subsequently on a highly sensitive MCT detector.

CAPILLARY ZONE ELECTROPHORESIS



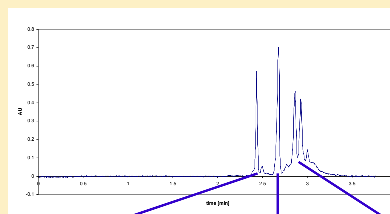
The principle of capillary electrophoresis (CE) lies only in applying voltage, generating an electro-osmotic flow (EOF) and separating the analytes due to different electrophoretic mobilities. As the EOF is higher than the mobility of all analytes anions and cations can be separated in the same run.

View of the whole setup including high voltage supply, beam condenser and spectrometer

Close - up of the IR cell on the beam condenser

RESULTS

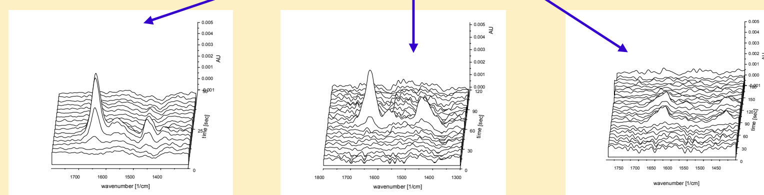
The figure right shows a typical electropherogram illustrating the separation of myoglobin, α - lactalbumin and β - lactoglobulin (two isoforms) recorded with a conventional UV detector placed immediately after the IR cell. The concentrations used were 4 g/L.



Conditions for the CE separation:

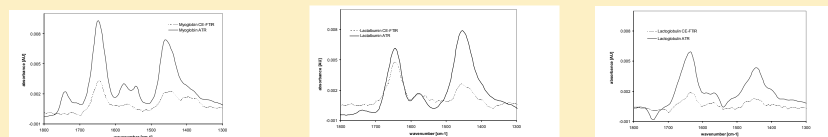
- Capillary: 50 µm id, uncoated fused silica, 60 cm length (40/20/5)
- Buffer: 20 mM borate, pH 10.0, adjusted with NaOH
- Separation voltage: +20 kV (22 µA) at injection end
- Detection: UV 214 nm
- Injection: siphoning 20 sec., 27 cm (injection volume 10 nL)

3D FT - IR stack plots



3D stack plots of FT - IR spectra recorded during the same run. The peak shapes represented in the UV electropherogram are clearly confirmed in the recorded FT - IR spectra regarding intensity and sharpness.

Comparison with reference spectra



Comparison of IR spectra recorded during a CE run and spectra of the pure substances obtained from ATR measurements confirming the proper performance of the CE – FT - IR setup and showing again very well the strong shifts of the wavenumbers according to the protein structure.

REFERENCES

[1] M. Kölhed, P. Hinsmann, P. Svasek, J. Frank, B. Karlberg and B. Lendl
"On-Line Fourier Transform Infrared Detection in Capillary Electrophoresis"
Anal. Chem. 2002, 74, 3843-48

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CONCLUSION

The results presented here describe the first hyphenation of CE and IR for the detection of proteins. The results presented show that the analysis of the secondary structure of proteins is feasible using this technique. This hyphenated system offers real - time, non - destructive and structure - specific information. Applications range from fast screening to giving complementary information where other techniques have difficulties to arrive at this information.

Another field of application would be protein analysis using capillary electrochromatography or to apply sample pretreatment steps using sequential injection to enhance sensitivity and selectivity of the separation.