INTRODUCTION

Here we present the on-line hyphenation between capillary electrophoresis and FTIR [2] applied to the study of the secondary structure of proteins. FTIR offers the advantage of real-time, non-destructive and molecule-specific detection as compared to UV, LIF or MS. Therefore FTIR 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 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).

RESULTS

The figure right shows a typical electropherogram illustrating the separation of myoglobin, α-lactoglobulin and β-lactalbumin (two isoforms) recorded with a conventional UV detector placed immediately after the IR cell. The concentrations used were 4 gL⁻¹.

3D FT-IR stack plots

Comparison with reference spectra

REFERENCES


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 molecule-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 electrophoresis or to apply sample pretreatment steps using sequential injection to enhance sensitivity and selectivity of the separation.