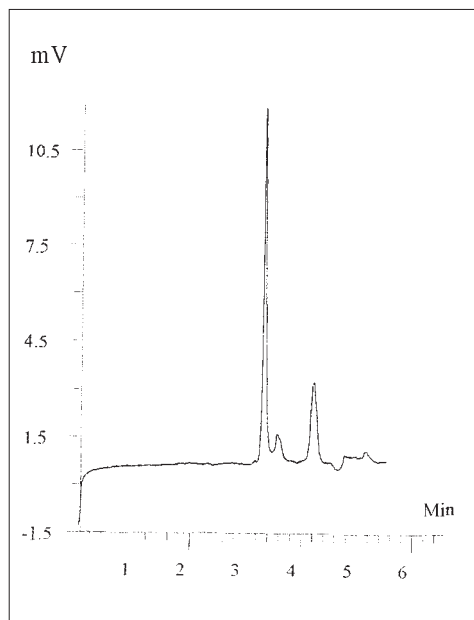


- Short Analysis Time
- Simple, Easy, Fast

CYTOCHROME C



Method Conditions:

Voltage: 10kV
 Capillary: MicroSolvCE Bare Fused Silica 75µm 39cm Total, 8.5cm Effective
 Injection: Hydrodynamic, 7mbar, 5 seconds
 Run Buffer: CElixir Accelerator (B) Solutions Mixed to pH 7.0
 Organics Added: None
 Detection: UV 214nm

Method:

Using a Microanalytical CE-211 Series Capillary Electrophoresis System (MO, USA) and a MicroSolvCE 75µ bare fused silica capillary, the separation of Cytochrome C and an impurity was easily accomplished. Mixing two different CElixir Accelerator Solutions (pH 6.2 and 8.2), the resulting BGE/run buffer pH was 7.0. The Cytochrome C sample (0.5 mg/ml) was dissolved in the CElixir pH 7.0 solution and injected directly onto the capillary after it was conditioned according to the CElixir Operating and Trouble Shooting Manual. A 5 second injection was made. The separation was completed within five minutes after injection.

Discussion and Rationale:

The pH of the BGE/run buffer was adjusted to 7.0 because at this pH (a very common biological pH), the Cytochrome C would be positively charged and this pH allows for good solubility of the protein and the associated impurity. The first peak in the electropherogram is Cytochrome C, the second is an impurity and the third is a neutral marker.

Comments:

When the sample was analyzed in 25mM phosphate pH 7.0 buffer and injected onto the same type of capillary with the same conditions, no peak could be seen. Increasing the strength to 100mM phosphate pH 7.0, a very broad peak (5000 Theoretical Plates) could be seen. With CElixir the above electropherograms can be seen.

Dr. Tiansong Wang
 Professor of Chemistry
 Peking University
 Beijing, China

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1 Analis Patent No. 5,611,903



MicroSolv Technology Corporation

101 Brighton Avenue
 Long Branch, NJ 07740
 Voice: 1-732-229-3400
 Fax: 1-732-229-2403
 email: MicroSolv2@aol.com
 web: MicroSolvTech.com