

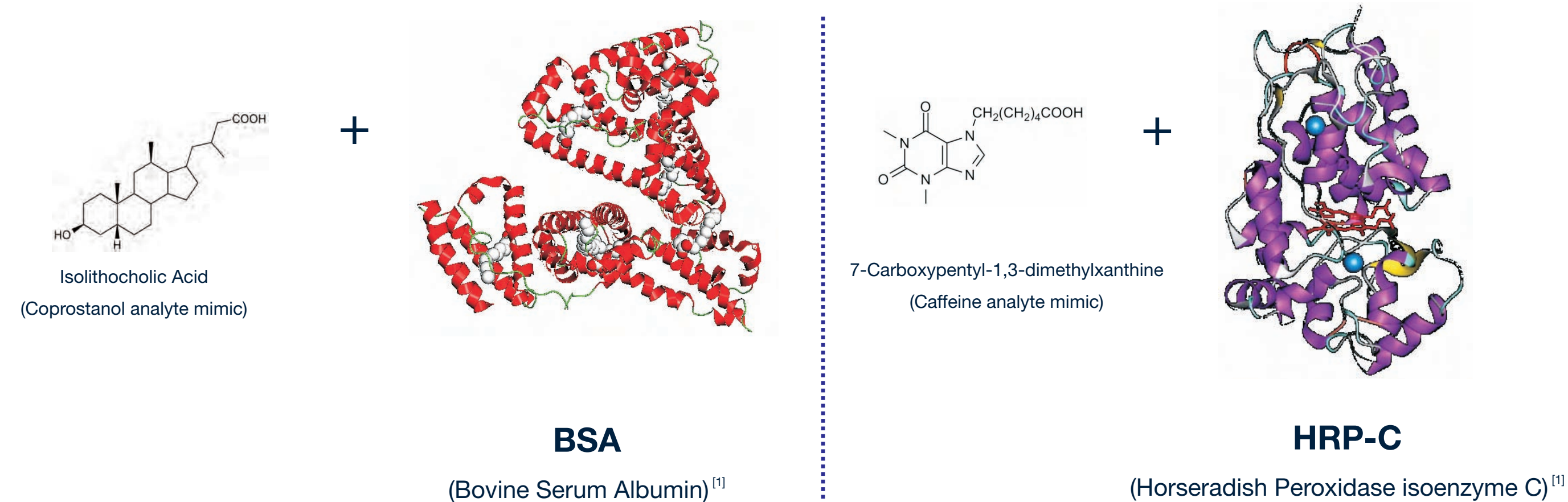
Characterization of Protein Conjugates by Capillary Zone Electrophoresis (CZE) Using Dynamic Self-coating Materials

H. S. Pecher, J. J. Carvalho, R. J. Schneider*, M. G. Weller, U. Panne

BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Str. 11, D-12489 Berlin

Introduction

▶ Bovine Serum Albumin (BSA) and Horseradish Peroxidase isoenzyme C (HRP-C) are two proteins widely used in immunoassays analytics. BSA is used as a carrier protein for antibody production directed to non-immunogenic haptens and HRP-C is used to synthesize enzymatic tracers, allowing sensitive detection after a substrate reaction.

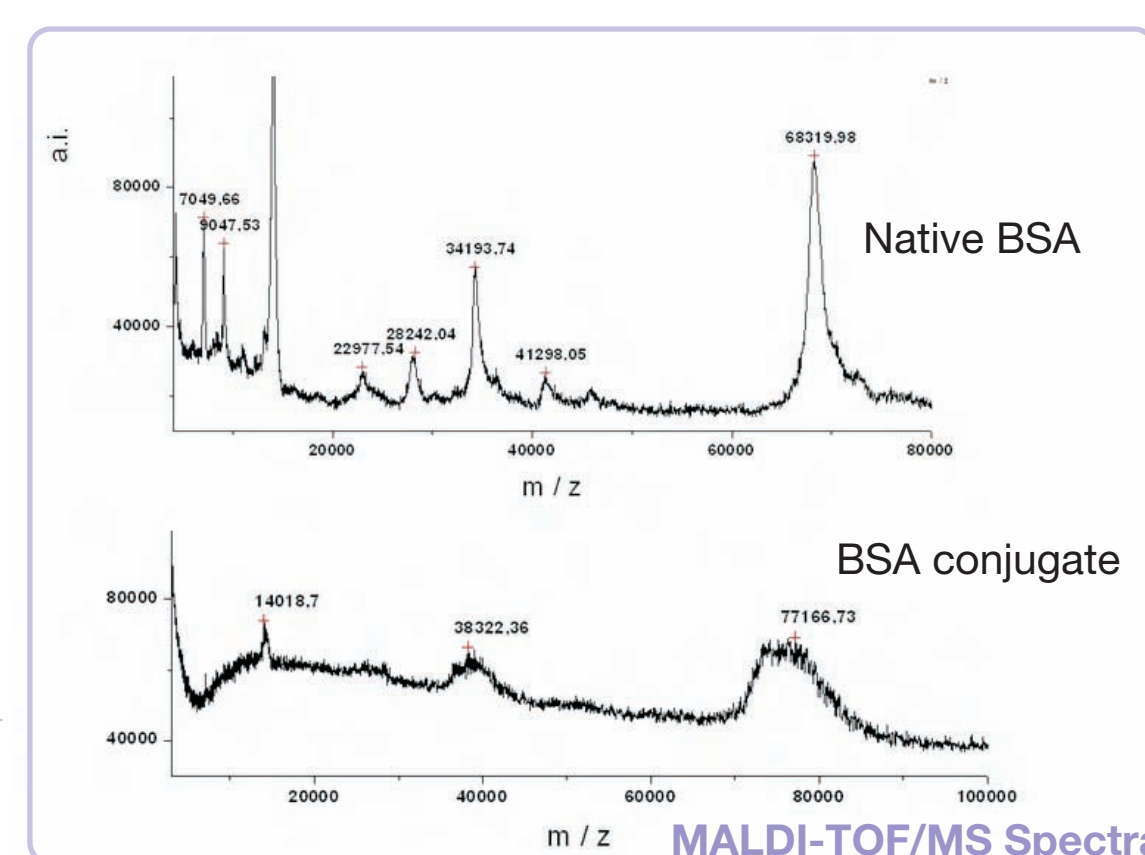


BSA contains 59 lysine residues but only 30 to 36 of them are available for coupling reactions.

In HRP-C, 3 to 4 lysine residues are available for coupling with the hapten.^[2]

▶ The molar coupling ratio (moles of hapten:moles of protein) in the final conjugates might influence the immunogenicity of an immunogen as well as tracer activity. Such relations are hitherto not thoroughly established due to a lack of suitable characterization techniques for those conjugates.

▶ MALDI-TOF/MS and LC-MS/MS experiments showed but a broad interval of masses thwarting complete elucidation of the conjugate coupling density.



Objective

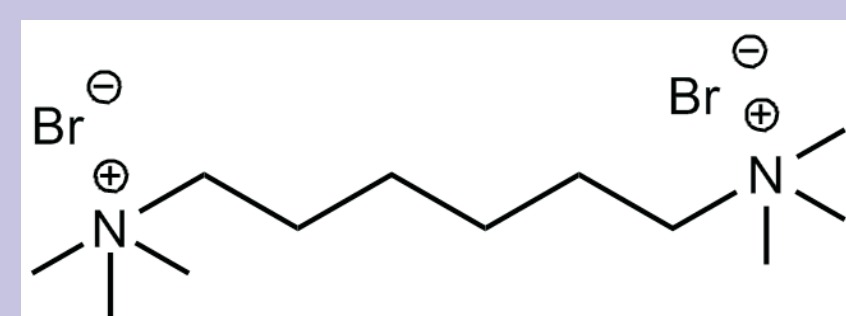
Optimize CZE for characterization of protein conjugates using dynamic self-coating materials.

Experimental

▶ Additives used as dynamic self-coating materials

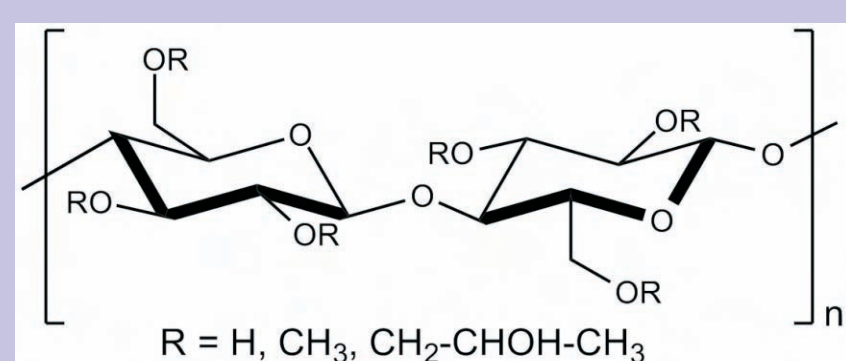
Hexamethonium Bromide (HMB)

- ◆ Quaternary ammonium alkane
- ◆ Concentrations: 0.3 mM - 1 mM^[3]
- ◆ Adsorption via hydrogen bonding and electrostatic interactions^[4]



Hydroxypropylmethylcellulose (HPMC)

- ◆ Neutral hydrophilic polymer
- ◆ Concentration: 0.25 %
- ◆ Adsorption via hydrogen bonds^[4]



▶ Instrumentation

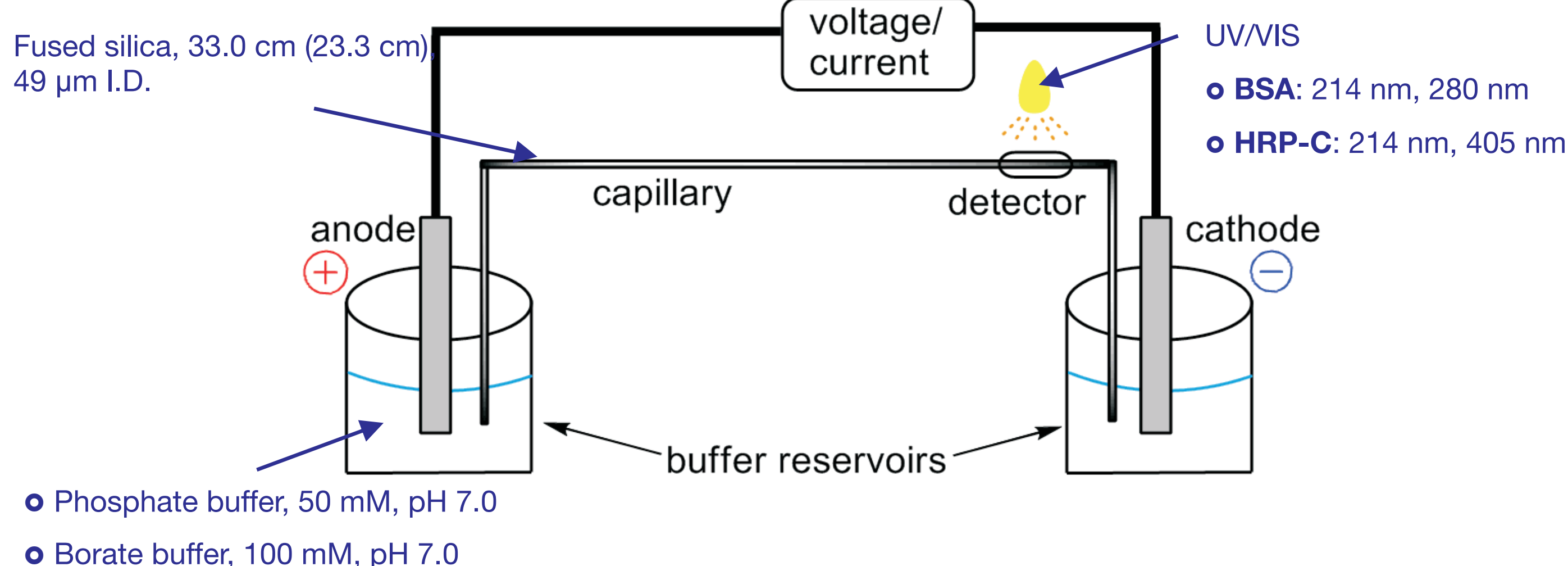


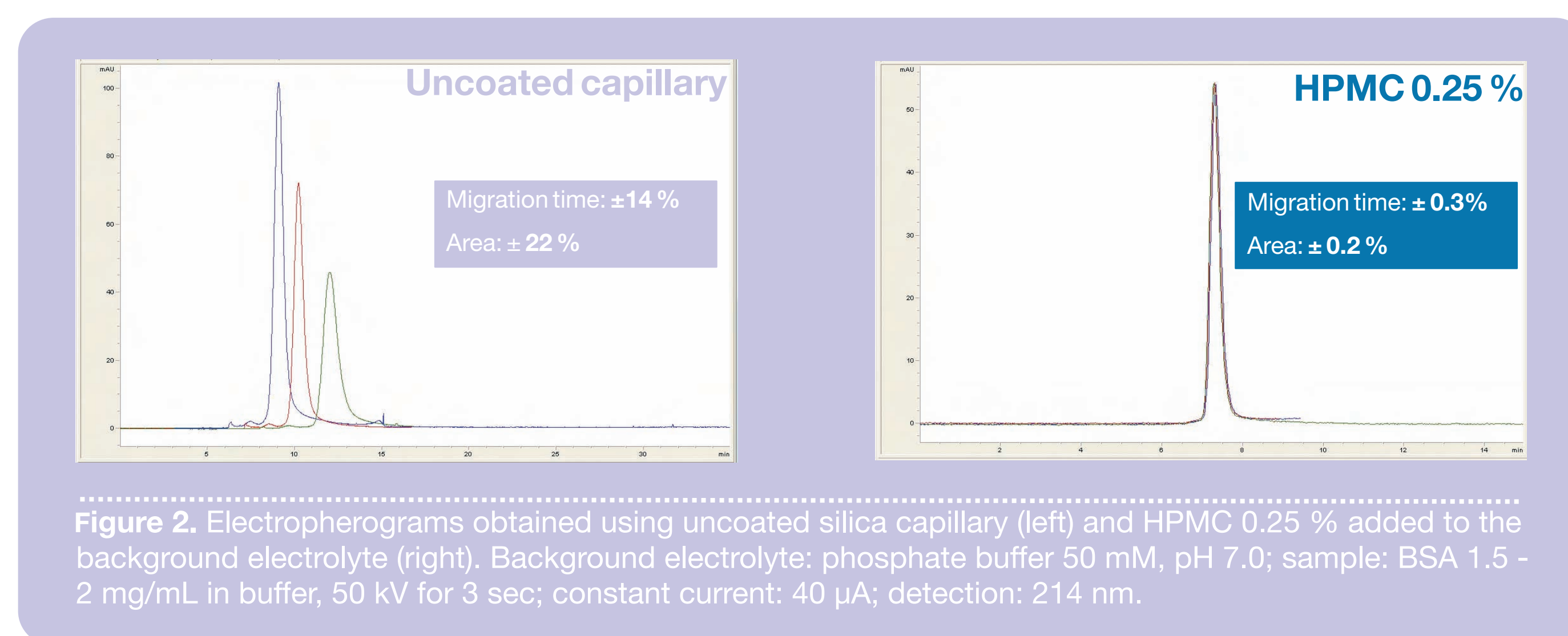
Figure 1. Capillary Electrophoresis Instrumentation (HP 3DCE station)

Literature

- [1] Swiss-Prot database (<http://www.expasy.ch/>), Swiss Institute for Bioinformatics, 2003. Structures downloaded on 2008-11-10.
- [2] N.C. Veitch, *Phytochemistry*, 2004, 65, 249-259.
- [3] R.P. Oda, B.J. Madden, T.C. Spelsberg, J.P. Landers, *J. Chromatogr. A*, 1994, 680, 85-92.
- [4] D. Corradini, *J. Chromatogr. B*, 1997, 699, 221-256.

Results and Discussion

▶ HPMC as dynamic self-coating material



▶ HMB as dynamic self-coating material

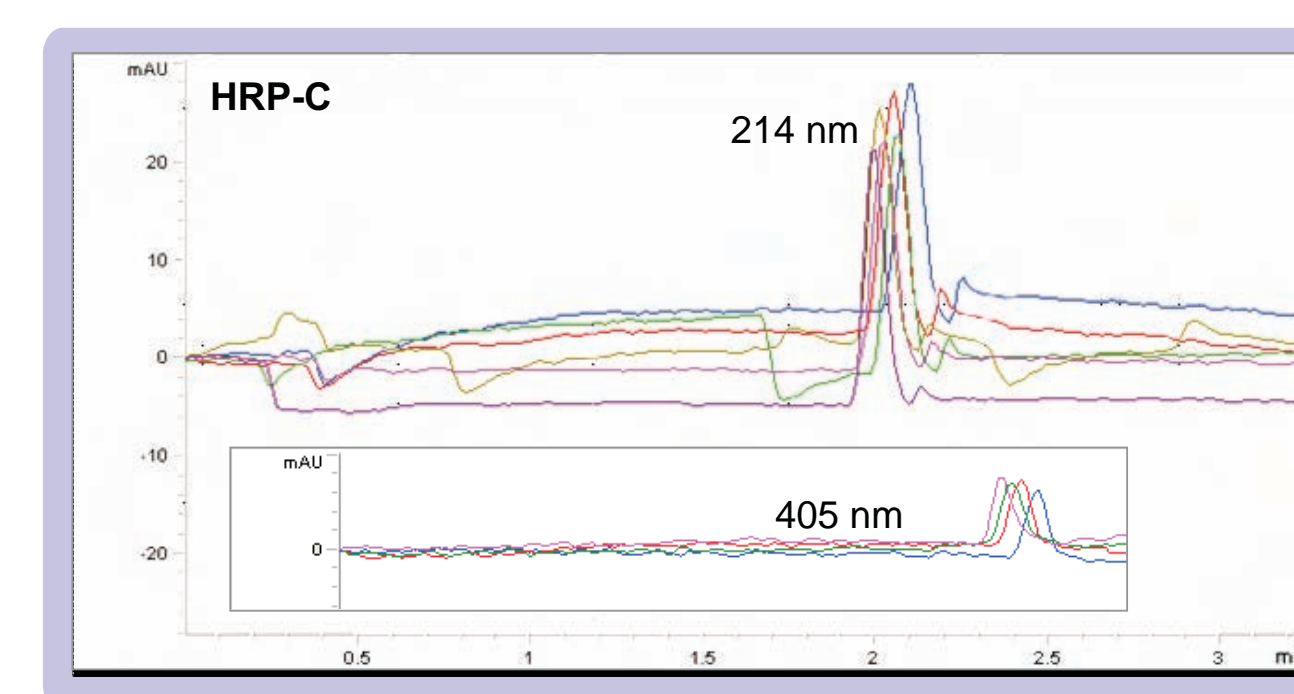


Figure 3. Electropherograms obtained using HMB 1.0 mM as dynamic coating. Background electrolyte: borate buffer 100mM, pH 7.0; applied voltage: 25 kV; sample: 1 mg/ml HRP-C in buffer, 30 kV for 5 sec. Migration time (2.04 min) reproducibility: ±1.7 %, area reproducibility: ±2.6 %.

▶ BSA and HRP-C conjugates

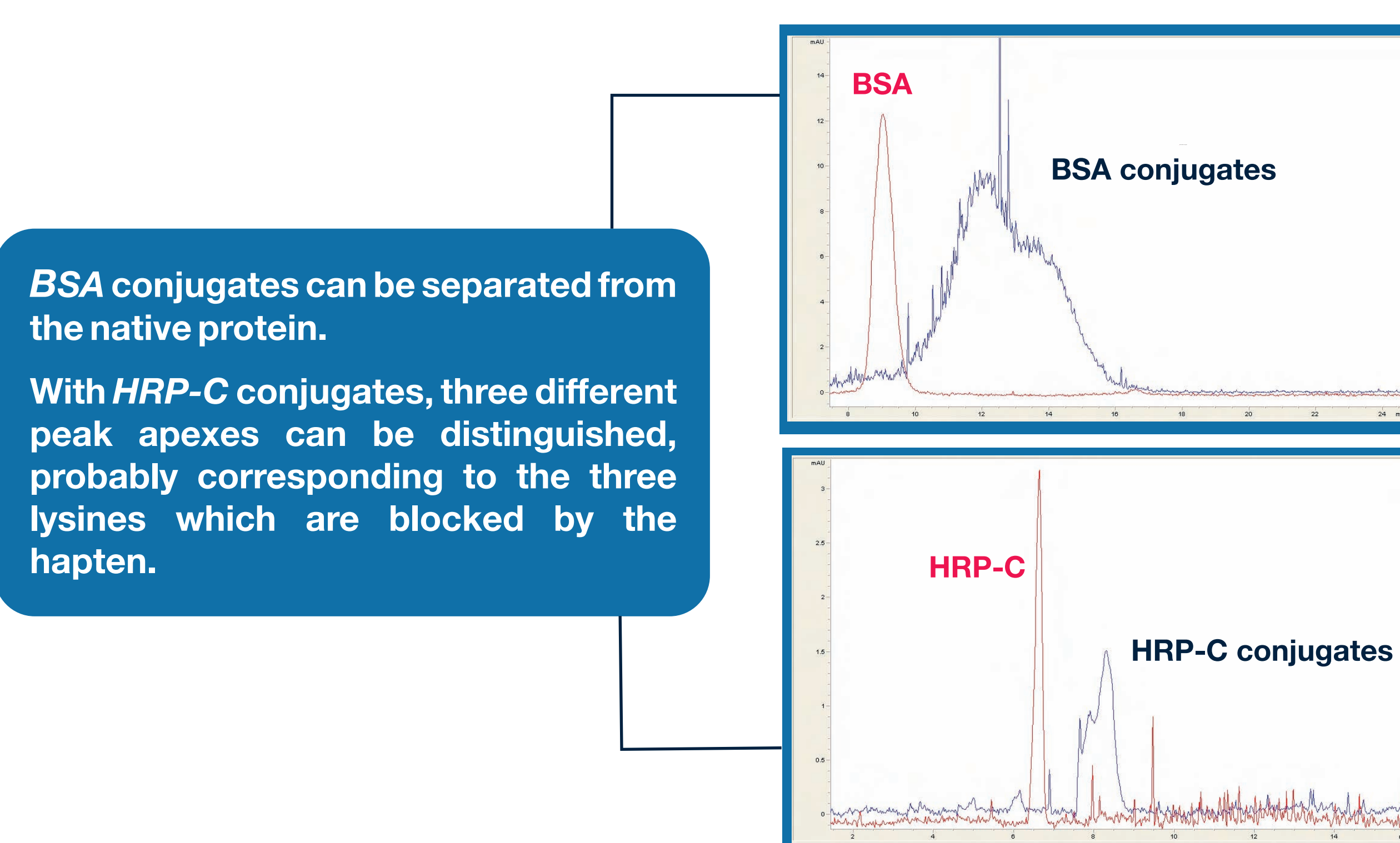


Figure 4. Electropherograms obtained when HPMC 0.25 % was added to the background electrolyte (phosphate buffer 50 mM, pH 7.0); Injection: 50 kV for 3 sec; constant current: 40 µA, detection: 214 nm (BSA), 405 nm (HRP-C).

▶ Although HMB (1 mM) leads to reproducible peak areas and migration times, the short migration times (not enough time inside the capillary) makes it inappropriate for protein conjugate separation.

▶ The separation of protein conjugates from the native protein was achieved by adding HPMC 0.25 % to the running background electrolyte (phosphate buffer 50 mM, pH 7.0).

Outlook

▶ The separation of the conjugate peaks will be carried out using different background electrolytes.

▶ Isoelectric focusing (IEF) capillary electrophoresis will be tried out using HPMC as dynamic coating.

Acknowledgement

We would like to thank WG Immunochemical Methods co-workers A. Lehmann for technical assistance with the CE instrument and S. Flemig for the MALDI-TOF/MS measurements.