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A simple, rapid, and sensitive method for analysis of SYPRO Red labeled sodium dodecyl sulfate-protein complexes by capillary electrophoresis with laserinduced fluorescence

We describe a segmental filling method for the analysis of SYPRO Red labeled sodium dodecyl sulfate (SDS)-proteins (SRSPs) by capillary electrophoresis-laser induced fluorescence (CE-LIF) with electroosmotic counterflow of poly(ethylene oxide) (PEO). It is shown that SDS and salt play a crucial role in determining the fluorescence intensity of the SRSP. Although the fluorimetric measurements reveal that the SRSPs fluoresce strongly in Tris-borate (TB) buffer containing 0.1% SDS and high concentrations of NaCl (100 mM), these conditions are not appropriate to CE in view of Joule heating. To overcome that impediment, we applied a plug of 0.1% SDS (1/5 to 1/3 of the injection volume) prior to injection of samples (0.64 μ L) prepared in TB buffer containing 50 mM NaCl and SYPRO Red. When using a background electrolyte of 0.6% PEO in TB buffer containing NaCl, electroosmotic counterflow of the analytes allows one to concentrate large sample volumes (up to 1/3 of effective capillary length) in 21 min, with detection of 0.35 and 0.10 nM for bovine serum albumin and casein, respectively. With a linear dynamic range from 10 nM to 5 μ M, this method provides the capability of determining the concentration of casein in cow's milk as 0.45 \pm 0.03 mM (n = 5).

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1 Introduction

Proteomics seeking to characterize the entire complement of proteins expressed in a cell or cell fraction under defined conditions, requires techniques with sensitivity, great efficiency, and high throughput [1–3]. Mass spectrometry in conjunction with 2-D PAGE or with liquid chromatography is commonly applied for that purpose [4, 5]. Supplementing those methods, capillary electrophoresis (CE) combined with laser-induced fluorescence (LIF), with high resolving power, sensitivity, rapidity, and potential for automation, has been shown capable of analyses of low amounts of proteins in the presence of abundant ones and of hydrophilic proteins in the presence of hydrophobic ones [6–13]. Using UV lasers such as the argon ion laser at 275 nm, limits of detection (LODs) for proteins

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Abbreviations: CAS, β -casein; CF, concentration factor; CON, conalbumin; HGB, hemoglobin; MC 540, merocyanine 540; OVA, ovalbumin; PEO, poly(ethylene oxide); SRSB, SYPRO Red labeled SDS-BSA; SRSP, SYPRO Red labeled SDS-proteins; TB, Tris-borate

at the nm level are readily achieved by CE, allowing for single-cell analysis [14, 15]. Alternatively, the analysis of proteins labeled with fluorophores or derivatized with a strongly fluorescent complexing agent can employ relatively low-cost and stable lasers such as argon ion and He-Ne lasers [16–19].

Since Cohen and Karger [20] reported the use of crosslinked polyacrylamide for the separation of proteins by CE, numerous polymer matrices including gels and polymer solutions have been tested with varying degrees of success [8, 21-24]. With a capability of dynamic coating of the capillary wall and a high sieving ability, poly(ethylene oxide) (PEO) has been particularly useful for protein analysis [25-28]. Recently, we have applied PEO solutions for the analysis of large-volume protein samples (up to 0.3 × effective capillary length) using an Nd-yttriumaluminum-garnet (Nd-YAG) laser at 266 nm [10, 26]. During concentration and separation, PEO enters the capillary filled with Tris-borate (TB) buffer by EOF. Negatively charged proteins migrate electrophoretically into the PEO solution from the sample zone in a direction opposite from that of EOF and thus decelerate and concentrate under the effects of molecular sieving and increased viscosity of the polymer solution.

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Based on our previous approaches [29, 30], we developed a technique for the analysis of large-volume proteins by CE-LIF using a He-Ne laser at 543.6 nm. Unlike using a UV laser, this method is cost-effective and versatile. Fast derivatization reactions of proteins with SYPRO Red or merocyanine 540 (MC 540) render these dyes suitable for the analysis of proteins in biological samples without tedious precolumn fluorescent labeling. The results show that SDS, salt, and ionic strength play an important role in determining detection sensitivity and resolution, in view of their effects on mobility, fluorescence, and adsorption onto the capillary wall.

2 Materials and methods

2.1 Equipment

The basic design of the CE separation system was that previously described [30]. Briefly, a high-voltage (constant voltage, 30 000 V, 1 mA) power supply (Gamma High Voltage Research, Ormond Beach, FL, USA; model No. PR30-1P) was used to generate the electric field. The entire detection system was enclosed in a black box with a high-voltage interlock. The high-voltage end of the separation system was placed in a laboratory-made plexiglass box for safety. A 1.5 mW He-Ne laser with 543.6 nm output from Uniphase (Mantence, CA, USA) was used for excitation. The light was collected with a $10 \times$ objective (numerical aperture = 0.25). One RG 610 cutoff filter (Edmund Industrial Optics, Arrington, NJ, USA) was used to block scattered light before the emitted light reached the photomultiplier tube (Hamamatsu R928, Shizuoka-Ken, Japan). The amplified currents were transferred directly through a 10 k Ω resistor to an A/D converter at 10 Hz (CSW 1.7; DataApex, Prague, Czech Republic) and stored in a personal computer. Bare fusedsilica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with 75 μ m ID and 365 μ m OD were used for protein separation without internal coating.

2.2 Chemicals

All chemicals for preparing buffer solutions and polymers were obtained from Sigma (St. Louis, MO, USA), except PEOs (M_r 4000000 and 8000000) which were from Aldrich (Milwaukee, WI, USA). MC 540 was obtained from Acros Organics (Geel, Belgium) and SYPRO Red from Molecular Probes (Eugene, OR, USA). TB buffers were prepared from tris(hydroxymethyl)aminomethane (Tris) by adjustment with boric acid to pH 8.0, 9.0, and 10.0. Unless otherwise noted, the molarity of Tris represents that for the TB buffer. PEO solutions were prepared in TB buffers, pH 9.0 [30]. Bovine serum albumin (BSA), β-casein (CAS), conalbumin (CON), hemoglobin (HGB), and ovalbumin (OVA) were dissolved in water and diluted to suitable concentrations with TB buffer, pH 9.0, prior to CE analysis. Some of their physical and chemical properties are listed in Table 1 [31]. Cow's milk was purchased from a grocery store.

2.3 On-line concentration and separation

Capillaries were washed with 0.5 M NaOH overnight. Prior to each run, they were subjected to electrophoresis in 0.5 M NaOH at 1.0 kV for 10 min to remove residual PEO solution and refresh the capillary wall. The sample was injected by hydrodynamic injection from a 30 cm height for a time ranging from 10 to 180 s or by electrokinetic injection at 15 kV for a time ranging from 5 to 120 s. If an SDS plug was applied (for details see below), it was injected prior to the sample. The anodic end of the capillary was then immersed in PEO solution. In application to SRSPs, the proteins were allowed to react with the fluorescent dye in 38 mM TB buffer, pH 10.0, containing 50 mM NaCl, and 0.1% SDS prior to their CE separation at 15 kV in a PEO solution, pH 9.0, containing 5.0 mM NaCl. In the case of labeling the proteins with MC 540, a 1.7% PEO (4 MDa) solution was prepared in 50 mm TB buffer, pH 8.0, containing 4.0 μ M MC 540. Protein solutions were prepared in 50 mm TB buffer, pH 9.0. During CE, PEO

Table 1. Chemical and	d physical	l properties of	proteins
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Protein (source)	Abbreviation	M _r	p/	Hydrophobicity ^{a)}
β -Casein (bovine milk)	CAS	24 500	4.7	N.F. ^{b)}
Ovalbumin (chicken egg)	OVA	45 000	4.6	980
Hemoglobin (bovine)	HGB	64 500	6.9–7.4	960
Bovine serum albumin (bovine plasma)	BSA	66 000	4.6	1000
Conalbumin (chicken egg white)	CON	78000	6.6	980

a) [31]

b) Not found

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solutions entered the capillary by EOF. Reaction between proteins, SDS, and MC 540 in applications where they are contained in the PEO solution took place during their electrophoretic migration through the counterflowing PEO solution. The crossing between electrophoretically migrating and EOF-driven particles results in on-line concentration concomitant with separation.

2.4 CE analysis of cow's milk

To reduce the adsorption of milk constituents onto the capillary wall, 0.5 mL of milk was mixed with 99.5 mL of 38 mM TB buffer, pH 10.0, containing 0.1% SDS and 50 mM NaCI. SYPRO Red stock solution ($5000 \times$) was added to the mixture to a concentration of $10 \times$. Hydrodynamic injection was carried out from a 30 cm height for 20 s, followed by CE separation at 15 kV.

3 Results and discussion

3.1 Effect of buffer concentration and ionic strength on fluorescence intensity of SYPRO Red labeled SDS-proteins

Over the last few years, a series of SYPRO dyes have been developed and tested for staining proteins separated by SDS-PAGE [32, 33]. These dyes interact with the SDS-protein complexes rather than protein itself; thus, SDS can be assumed to play a crucial role in determining the sensitivity of their fluorimetric detection. It has also been found that the ionic strength of the background electrolyte is an important parameter with respect to sensitivity of fluorimetric detection and to resolution [34]. The present study of the fluorimetric detection sensitivity and of the resolution of fluorescently labeled SDSproteins by CE-LIF provides a more detailed analysis of those dependencies since it was conducted by a technique of segmental filling of the capillary which allows one to separately investigate the effects of SDS and buffer ionic strength on fluorescence intensity in three different phases, viz. (i) capillary contents, (ii) PEO solution, and (iii) sample.

(i) Figure 1A shows that the fluorescence intensity of the SYPRO Red labeled SDS-BSA (SRSB) decreased with increasing concentration of TB buffer, pH 10.0, in the capillary through which CE proceeds with electroosmotic counterflow generated by a solution containing 0.6% PEO, 50 mm TB, pH 9.0, 5 mm NaCl. It should be noted that the fluorescence intensities of SRSB are only slightly different (<1.0%) at pH values between 7.0 and 10.0, while adsorption of PEO on the capillary wall is stronger at pH values less than 9.0 [29]. Although we have shown



Figure 1. Effects of TB and NaCl concentrations on the fluorescence intensity of SRSB. The labeled SDS-BSA (1 µM) sample was prepared in 38 mM TB, pH 10.0, containing $2 \times$ SYPRO Red and 0.1% SDS and applied electrokinetically as described in Section 2.3. Separations were conducted at 250 V/cm. Capillary, 60 cm in total length, 50 cm in effective length. (A) TB buffer, pH 10.0, at various concentrations filled into the capillary; PEO, 0.6% (8 MDa), prepared in 50 mm TB, pH 9.0, containing 5.0 mM NaCl, is introduced into the capillary by EOF. (B) 0.6% PEO (8 MDa) prepared in TB buffer, pH 9.0, at various concentrations, containing 5.0 mm NaCl; the capillary contains 400 mM TB buffer, pH 10.0. (C) NaCl concentration in the solution containing 0.6% PEO (8 MDa) prepared in 50 mm TB buffer, pH 9.0, is varied. The capillary contains 400 mM TB buffer, pH 10.0.

that CE can be conducted reproducibly at concentrations of TB buffer over 1.0 M [10], which are needed in order to minimize PEO adsorption onto the inner capillary wall and thus to achieve fast and reproducible protein separations, the resulting decrease in fluorescence is obviously problematic. The decrease in fluorescence appears to be mainly due to quenching caused by Tris, a notion supported by fluorimetric measurements on Tris solutions of various concentrations (data not shown). In addition, Joule heating may contribute to the decrease of fluorescence intensity at high TB concentrations.

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(ii) Figure 1B shows that the fluorescence intensity of the SRSB complex varies in biphasic fashion with the TB concentration in the PEO solution, pH 9.0, with a sharp decrease in fluorescence beyond 75 mM when the capillary contains TB, pH 10.0, at a constant concentration of 400 mM. Figure 1C depicts the impact of NaCl concentration in the PEO solution on the fluorescence intensity of SRSB in CE when the capillary is filled with 400 mM TB buffer, pH 10.0, revealing that a small amount of NaCl up to 5 mM in the PEO solution effectively augments the fluorescence intensity of SRSB while higher NaCl concentrations inhibit it [35]. The presence of a small amount of salt is also of benefit for minimizing protein adsorption onto the capillary wall [36].

(iii) Although SRSB fluoresces strongly at a steady-state concentration of 0.5% SDS, we have found that large amounts of SDS in the sample cause the deterioration of fluorescence and resolution presumably due to Joule heating. Figure 2A shows that the fluorescence intensity of SRSB complexes is maximized when the protein sam-



Figure 2. Effects of (A) SDS and (B) NaCl concentrations in the sample on the fluorescence intensity of SRSB. The SRSB (1 μ M) sample was prepared in 38 mM TB, pH 10.0, containing 2 × SYPRO Red and varying concentrations of SDS and NaCl, and was applied electrokinetically. The capillary was filled with 400 mM TB buffer, pH 10.0. A solution of 0.6% PEO (8 MDa) prepared in 50 mM TB buffer at pH 9.0 containing 5 mM NaCl entered the capillary by EOF. Other conditions were as described in Fig. 1.

ple is prepared in TB buffer containing approximately 0.1% SDS. Figure 2B shows that the fluorescence of the SRSB increased with increasing NaCl concentration in the sample phase, suggesting that the Coulombic interaction between SDS-proteins and SYPRO Red should not be a major contributor to formation of the complex. Together with the effect of SDS concentration on fluorescence of the complex these results support the notion that the interaction between fluorescent dye and protein is through the hydrophobic tail of SDS, i.e., hydrophobic regions or patches formed by association of those tails. Thus, maximal sensitivity of CE for the analysis of SRSB under the discontinuous conditions described in the present report is reached in a capillary filled with 400 mM TB, pH 10.0, with EOF of a PEO plug prepared in 50 mm TB, pH 9.0, containing 5 mM NaCl, and a protein sample prepared in 38 mM TB, pH 10.0, containing 0.1% SDS, 50 mM NaCl, and $2 \times$ SYPRO Red.

3.2 Effect of an SDS plug on the CE of fluorescently labeled SDS-proteins

Although the sensitivity was optimized using the condition listed above, separation of proteins under those conditions is unsuccessful, mainly due to changes in the electrophoretic mobility of the proteins in the presence of SDS. The separation might be possible using 8% linear polyacrylamide gel [18], however, it requires on-column polymerization, shortening the life time of the capillary and giving rise to irreproducibility. To overcome this impediment, we applied an SDS plug prior to sample injection, which has been shown previously to be an effective way for improving the separation efficiency of proteins [28]. Figure 3 shows the effect on resolution of SRSPs using plugs of 1.0% SDS for 10 s (Fig. 3A) and 0.1% SDS for 60 s (Fig. 3B). It should be noted that the critical micellar concentration (CMC) for SDS in aqueous solution is about 0.23% [37]. At 1.0% SDS, the peaks appearing in the electropherogram around 10 min appear to be free dye detected due to an increase in the dye fluorescence after binding to SDS micelles. Owing to the formation of protein complexes with micellar SDS, the migration times were slightly prolonged in the presence of 1.0% SDS. Although the peaks were not completely resolved at baseline, the SDS plugs produce a significant increase in resolution and reproducibility. For example, the resolution value between OVA and HGB was 0.01 in the absence of an SDS plug, 0.71 on application of a 1.0% SDS plug, and 0.91 after application of a 0.1% SDS plug, respectively. The relative standard deviation (RSD) values for the migration time of HGB are less than 1.2% and about 2.5% in the presence and absence of the SDS plugs, respectively. Table 2 further shows the LODs at the signal-to-noise

1.0% SDS				0.1% SDS					
CON	BSA	CAS	OVA	HGB	CON	BSA	CAS	OVA	HGB
13.19	13.69	13.89	13.99	14.05	12.27	12.84	13.03	13.13	13.19
(0.78%)	(0.85%)	(0.93%)	(1.01%)	(1.15%)	(0.73%)	(0.81%)	(0.88%)	(0.95%)	(1.06%)
0.09	0.05	0.03	0.03	0.06	0.09	0.08	0.05	0.03	0.04
⁾ 1.11	3.66	9.62	9.76	3.04	0.96	1.22	3.31	8.60	6.03
60.4	45.3	30.1	78.8	12.4	28.6	8.69	5.67	1.62	5.22
)	CON 13.19 (0.78%) 0.09 1.11 60.4	13.19 13.69 (0.78%) (0.85%) 0.09 0.05 1.11 3.66 60.4 45.3	1.0% SDS CON BSA CAS 13.19 13.69 13.89 (0.78%) (0.85%) (0.93%) 0.09 0.05 0.03 1.11 3.66 9.62 60.4 45.3 30.1	1.0% SDS CON BSA CAS OVA 13.19 13.69 13.89 13.99 (0.78%) (0.85%) (0.93%) (1.01%) 0.09 0.05 0.03 0.03 1.11 3.66 9.62 9.76 60.4 45.3 30.1 78.8	1.0% SDS CON BSA CAS OVA HGB 13.19 13.69 13.89 13.99 14.05 (0.78%) (0.85%) (0.93%) (1.01%) (1.15%) 0.09 0.05 0.03 0.03 0.06 1.11 3.66 9.62 9.76 3.04 60.4 45.3 30.1 78.8 12.4	1.0% SDS CON BSA CAS OVA HGB CON 13.19 13.69 13.89 13.99 14.05 12.27 (0.78%) (0.85%) (0.93%) (1.01%) (1.15%) (0.73%) 0.09 0.05 0.03 0.03 0.06 0.09 1.11 3.66 9.62 9.76 3.04 0.96 60.4 45.3 30.1 78.8 12.4 28.6	1.0% SDS 0. CON BSA CAS OVA HGB CON BSA 13.19 13.69 13.89 13.99 14.05 12.27 12.84 (0.78%) (0.85%) (0.93%) (1.01%) (1.15%) (0.73%) (0.81%) 0.09 0.05 0.03 0.03 0.06 0.09 0.08 1.11 3.66 9.62 9.76 3.04 0.96 1.22 60.4 45.3 30.1 78.8 12.4 28.6 8.69	1.0% SDS 0.1% SDS CON BSA CAS OVA HGB CON BSA CAS 13.19 13.69 13.89 13.99 14.05 12.27 12.84 13.03 (0.78%) (0.85%) (0.93%) (1.01%) (1.15%) (0.73%) (0.81%) (0.88%) 0.09 0.05 0.03 0.06 0.09 0.08 0.05 1.11 3.66 9.62 9.76 3.04 0.96 1.22 3.31 60.4 45.3 30.1 78.8 12.4 28.6 8.69 5.67	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Table 2. Effect of the SDS plug on the mobility, bandwidth, plate number and LOD values for proteins

a) Data were taken from Fig. 3.

b) Conditions were as in Fig. 3.



Figure 3. Separations by CE of five SRSPs in 1.5 M TB, pH 10.0, with counterflow by EOF at 375 V/cm of a 1.7% PEO (4 MDa) solution prepared in 400 mM TB, pH 9.0, containing 5.0 mM NaCl. SRSPs were prepared in 38 mM TB, pH 10.0, containing 0.1% SDS, 50 mM NaCl, and $10 \times$ SYPRO Red. An SDS plug was applied prior to sample injection from a 30 cm height for 20 s. The composition of the plug was (A) 1.0% SDS applied for 10 s and (B) 0.1% SDS for 60 s. Peak identities and sample concentrations (nM): (A) 1, CON (700); 2, BSA (800); 3, CAS (500); 4, OVA (300); 5, HGB (700). (B) 1, CON (600); 2, BSA (300); 3, CAS (200); 4, OVA (100); 5, HGB (400).

ratio (S/N) = 3 are at the nM level when a 0.1% SDS plug was applied [28]. Compared to the 0.1% SDS plug, a 1.0% SDS plug provided higher LOD values, apparently because of a relatively unstable baseline. When compared to Harvey's result [18], this method is less sensitive, presumably because of a higher background and shift in the baseline, but is slightly faster due to a high EOF.

3.3 Concentration of protein under the CE peaks

Recently, two on-line concentration techniques based on pH and viscosity discontinuities as well as on a sieving mechanism for the CE analysis of SDS-proteins have been reported, yielding sensitivity improvements greater than 1500-fold [10, 26]. In short, that concentration effect was achieved by injection of an SDS plug (in the cathodic side of the sample zone) prior to the sample into a TB solution at pH 10.0 with concurrent electroosmotic counterflow of a PEO solution at pH 9.0. After injection of the sample (pH 10.0), the proteins reacted with SDS contained in the plug (pH 10.0), with a concomitant increase in mobility, before entering into the PEO solution at pH 9.0, where they concentrated due to deceleration at the lower pH, due to the accumulation at a restrictive polymeric surface with sieving properties and high viscosity, and due to the opposition of EOF to mobility. In fact, the accumulation at a concentrated zone of the proteins accelerated through SDS-binding at the interface to a PEO solution can be viewed to be analogous to the formation of concentrated sample zones at the surfaces of gels, zones which also narrow in proportion to mobility (field strength). Table 3 shows the peak concentration achieved, with the concentration factors (CFs) ranging from 25 to 58. The LOD values for the SRSPs are reduced to 0.84 nm-28 pm when CE is carried out in the 40 cm capillary filled with 1.5 M TB buffer, pH 10.0. The definition of CF is the ratio of the LOD obtained for the injection at 30 cm for 10 s to that for 180 s. When a 60 cm capillary filled with 400 mm TB buffer, pH 10.0, was applied to the CE of SRSPs, the LODs were reduced to pm, with the CF values increased to 78-fold (LODs obtained at 15 kV for 5 s compared to those for 120 s). The LOD of 26 pM for BSA shows that this method is slightly more sensitive than Harvey's [18]. However, the loss in resolution is problematic, showing the importance of an SDS plug. It is important to note that the fluorescence intensities for the protein complexes

 Table 3. Comparison of migration time, LODs, and CF under different separation conditions

	A ^{a)}			B ^{b)}			
	Migration time (RSD)	LOD (пм)	CF	Migration time (RSD)	LOD (пм)	CF	
CON	20.27 (2.41%)	0.84	34	10.41 (2.13%)	0.036	48	
BSA	20.42 (2.97%)	0.35	25	10.43 (2.18%)	0.026	78	
CAS	20.51 (3.04%)	0.10	55	10.44 (2.20%)	0.033	59	
OVA	20.55 (2.89%)	0.028	58	10.47 (2.22%)	0.027	72	
HGB	20.58 (3.20%)	0.14	38	10.50 (2.25%)	0.029	45	

- a) 0.1% SDS plug was applied at 30 cm height for 60 s prior to sample injection; sample injection was conducted at 30 cm height for 180 s; the capillary was 40 cm (30 cm in effective length); other conditions were the same as in Fig. 3B.
- b) The capillary was filled with 400 mM TB, pH 10.0; 0.6% PEO (8 MDa) was prepared in 50 mM TB buffer containing 5 mM NaCl, pH 9.0; sample injection was conducted at 15 kV for 120 s; and the capillary was 60 cm (50 cm in effective length); other conditions were the same as in Fig. 3B.

increased with increasing injection volume over the range of 10–180 s at 30 cm height or of 5–120 s at 15 kV, with all R^2 values greater than 0.98. The relatively longer separation time when using PEO prepared in 400 mM TB compared to in 50 mM TB is due to a reduced EOF.

To illustrate the advantage of using SYPRO Red for fluorescent labeling of SDS-proteins in CE analysis, we compared the CE patterns with those using MC 540. MC 540 belongs to the family of benzoxazol merocyanine dyes with heterocyclic aromatic groups linked by a polymethine chain and has been commonly used for the analysis of membrane proteins [38, 39]. Separations of MC 540 labeled proteins cannot be conducted at high concentrations of TB buffer of high pH (> 8.0) since MC 540 is unstable under those conditions and fluoresces weakly. Figure 4 shows that the CE analysis of MC 540 labeled SDS-proteins is slow, the baseline is relatively unstable, and the peak profiles are relatively broad when compared to those using SYPRO Red. However, the dye is relatively less costly. When applying a plug of 0.15% SDS at 30 cm height for 90 s prior to injecting the protein sample at the same height for 180 s, the LOD values for SDS-CON, SDS-CAS, and SDS-HGB are only at the nm level. For SDS-BSA, MC 540 provides sensitivity similar to that of



Figure 4. Separation of four MC 540 labeled SDSproteins in the presence of EOF at 250 V/cm using 50 mM TB, pH 8.0, 1.7% PEO (4 MDa) containing 4.0 μ M MC 540. Capillary, 60 cm in total length, 50 cm in effective length, filled with 400 mM TB buffer, pH 10.0. A plug of 0.15% SDS was injected at 30 cm height for 90 s prior to hydrodynamic injection of a protein sample at the same height for 180 s. The concentrations of the proteins were 10 nM. Peak identities: 1, CON; 2, BSA; 3, OVA; 4, HGB.

SYPRO Red, presumably due to the fact that BSA is the most hydrophobic protein of the series under analysis as shown in Table 1.

3.4 Analysis of cow's milk

The electropherogram depicted in Fig. 5 shows that the analysis of a milk sample diluted with TB buffer was completed in 14 min. The last two peaks correspond to



Figure 5. CE-LIF separation of cow's milk proteins derivatized with SDS and fluorescently labeled by SYPRO Red, with electroosmotic counterflow by PEO solution. Peak identities: (1) unknown; (2) BSA; (3) CAS. Other conditions were as those described in Fig. 3B.

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BSA and CAS. The linear range for CAS is 10 nm – 5 μ M, with R^2 = 0.99. According to the linear regression analysis, we estimate a concentration of casein in cow's milk of 0.45 \pm 0.03 mM (n = 5). That result shows the potential of the method for monitoring the quality of milk, in addition to its advantages of low cost, operational simplicity, and robustness of the analysis.

4 Concluding remarks

We have demonstrated a simple concentration and separation method for the analysis of SDS-proteins by CE-LIF using a low-cost He-Ne laser. SYPRO Red reacts rapidly with proteins and its fluorescence intensity increases upon binding to an SDS-protein, an advantage when compared to fluorescent labeling with MC 540. The results shown in this study demonstrate that SDS and salt play an important role in determining detection sensitivity and resolution. The segmental filling technique allows the separation of proteins using low-viscosity polymer solution and noncovalent dyes, leading to simplicity, speed, and low costs. By applying a plug of SDS prior to sample injection, improved peak concentration has been achieved, presumably due to acceleration of protein migration prior to entering a counterflowing PEO solution of relatively high viscosity and sieving properties. The increased peak concentration translates into LODs of 0.35 and 0.10 nm for BSA and CAS, respectively.

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