ELSEVIER

Journal of Chromatography A, 699 (1995) 315–322

JOURNAL OF CHROMATOGRAPHY A

Thermo–optical absorbance detection of native proteins separated by capillary electrophoresis in 10 μm I.D. tubes

José María Saz[a], Beat Krattiger[b], Alfredo E. Bruno[b,*], José Carlos Díez-Masa[c], H. Michael Widmer[b]

[a]*Department of Analytical Chemistry, Faculty of Science, University of Alcalá de Henares, 28871 Alcalá de Henares, Madrid, Spain*

[b]*Corporate Analytical Research, Ciba-Geigy Ltd., 4002 Basle, Switzerland*

[c]*Institute of Organic Chemistry, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, Spain*

First received 17 November 1994; revised manuscript received 20 January 1995; accepted 20 January 1995

Abstract

Thermo–optical absorption (TOA) detection of native proteins separated by capillary electrophoresis is demonstrated in 10 μm I.D. tubes. The eluents were on-column *pumped* at 257 nm and *probed* by a hologram-based refractive index detector. The use of 10 μm capillaries allowed fast 2-min separations. Slower separations in wider tubes led to limits of detection (LODs) of $7 \cdot 10^{-9}$ *M* for bovine serum albumin. These LODs are comparable to those obtained with laser induced fluorescence and two orders of magnitude lower than in absorbance detection. Since native fluorescence of proteins is rare, TOA detection appears as a more universal detection scheme and thus suitable for other proteins having smaller amounts of fluorescent amino acids.

1. Introduction

The assay of proteins at trace levels (i.e., 10^{-8} *M*) is becoming a major issue in areas such as allergic diseases [1], preparation and quality control [2] of biopharmaceutical products obtained by DNA recombinant techniques. Immunoassays (e.g. radioimmunoassay or enzyme-linked immunosorbent assay), the analytical techniques usually employed to carry out such analysis, are labour-intensive, time-consuming, costly, and in some cases they lack of specificity and produce a non-neglectable amount of false positive results [1]. Therefore, the development of easy, fast, cost-effective, and sensitive analytical methods to solve those challenging problems is of interest. Although capillary electrophoresis (CE) has a great potential in protein separation, the sensitivity is limiting its application when performing trace analysis. UV absorption using commercially available detectors is rather insensitive and the limits of detection obtained for proteins are in the 10^{-6} *M* range [3]. On-column sample preconcentration to increase sensitivity using field amplification [4], chromatographic adsorption [5] or isotachophoresis [6] have a lot of potential but are somewhat difficult to implement for samples in complex matrices.

Swaile and Sepaniak [7] demonstrated CE of proteins with native laser-induced fluorescence (LIF) detection using as excitation wavelength

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00095-X

the frequency-doubled argon ion laser output at 257 nm. They reported a limit of detection (LOD) of 10^{-8} *M* for conalbumin. Lee and Yeung [3] obtained a higher sensitivity using a fundamental Ar^+ laser line at 275 nm which is closer to the absorption maximum of tryptophan and tyrosine. A LOD in the order 10^{-10} *M* for conalbumin and bovine serum albumin (BSA) was thus achieved. Most recently, a pulsed UV excimer laser operating at 248 nm has been also applied on native LIF detection in CE for conalbumin and BSA and the LODs obtained [8] are in the order of 10^{-9} *M*. As these authors showed, native LIF detection is highly sensitive but it is only applicable to those proteins having a high level of tryptophan and/or tyrosine amino acid residues at the protein surface. Since these residues are generally not abundant in proteins, only a few, such as those mentioned, display native fluorescence and therefore native LIF detection is only applicable to these special cases.

Derivatization procedures using highly fluorescent tags like fluorescein isothiocyanate (FITC) or *o*-phthalaldehyde (OPA) are a rather problematic approach. Pre-column derivatization can produce several association compounds between a given protein and the labelling agent leading to peak degradation and broadening [7]. LODs in the range of 10^{-10} *M*, using FITC as fluorescent tag in pre-column labelling of proteins, have been reported for conalbumin [7]. On-column tagging methods are an interesting way to overcome peak broadening. A fluorescent tag which interacts in a fast equilibrium with proteins is added to the buffer. This procedure, however, causes an increase in baseline noise which degrades the LODs. A 10^{-7} *M* LOD has been reported for BSA using toluidine naphthalene sulphonate (TNS) in the buffer [7]. Finally, post-column derivatization complicates the analysis and require a dedicated instrumental set-up; the reported LOD for myoglobin using this approach with OPA is in the 10^{-8} *M* range [9].

Capillaries whose I.D.s range between 25 and 75 μm were used to obtain the above-mentioned results using LIF detection. Faster analysis, higher efficiencies and resolutions could be obtained if the column I.D. is reduced, but then detection sensitivity is compromised because absorption and fluorescence depend on the optical path through the cell. Besides, the requirement for trace analysis arises an additional demand to develop detection schemes with increased sensitivity.

An alternative for sensitive detection of proteins would be thermo–optical absorption (TOA). In TOA detection [10–16] an intense laser pulse irradiates the sample repeatedly with a wavelength matching an absorption system in the sample. The amount of absorbed light, which is converted by radiationless transitions into heat, increases the solvent temperature in the illuminated region in a periodic fashion. As the refractive index (RI, n) of a material is a function of the temperature (T), those periodic changes in T manifest in changes in n (Δn) which can be conveniently monitored by a laser-based capillary RI detector and easily decoded with a lock-in amplifier. Because the recovered Δn signal is dependent, among other parameters, on the extinction coefficient of the sample, the output can be calibrated to produce quantitative measurements. No derivatization procedures are required if the sample happens to absorb at the wavelength of the pump laser used. This technique appears thus as an interesting option to preserve sensitivity if the capillary dimensions are reduced.

Previously, the high sensitivity of TOA detection was demonstrated in CE separations of samples labelled with highly absorbing tags [16], but, to the best of our knowledge, no data are available about TOA detection of native proteins. The objective of this article is thus to evaluate the use of TOA spectroscopy for detection of native proteins in CE separations using capillaries with I.D.s as small as 10 μm.

2. Experimental

2.1. TOA detector

The experimental set-up and operation principle of the TOA detector used in this study has

been recently described [15]. Fig. 1 shows a schematic of the TOA detector assembly which consisted of a hologram-based RI detector recently described [17]. The RI detector monitored the Δn produced when the sample was illuminated by the UV light from a frequency-doubled Ar^+ laser emitting at 257 nm (laser Model 2025-5, doubler Model 395 B; Spectra-Physics, Mountain View, CA, USA). To reduce the UV intensity noise to a value of less than 0.5%, a feed back system (Model 295, Spectra-Physics) was used. The UV pump beam (2 mm diameter) was modulated by a chopper (SR 540; Standford Research Systems, CA, USA) and focused by a quartz lens (No. 06 3010, Spindler & Hoyer, Göttingen, Germany) with $f = 16$ mm mounted on a three-dimensional translation stage (Model 3 MRN 03.5; Microcontrol/Newport). The numerical aperture (NA) was NA = 1/16 and as the

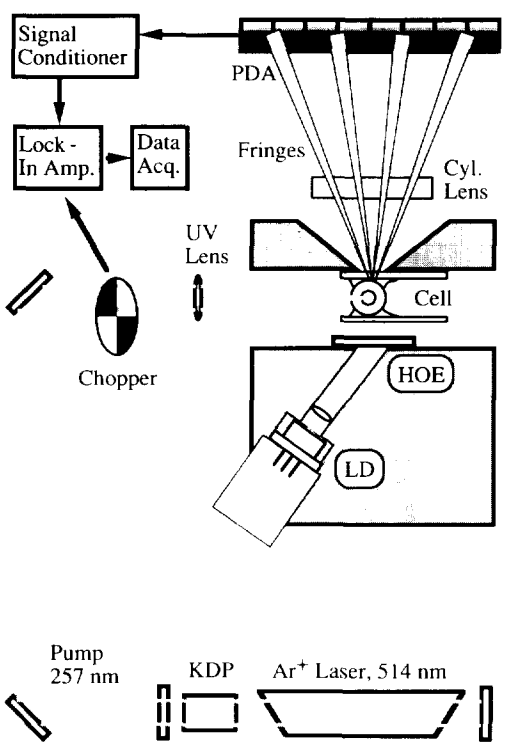

Fig. 1. Experimental setup for the TOA detector. KDP = Potassium dihydrogenphosphate frequency doubling crystal; LD = laser diode; HOE = holographic optical element; PDA = photodiode array.

rays crossed the capillary surfaces perpendicularly, the diameter of the pumping beam focus was $2w_0 = 2.6\ \mu m$ ($= 2\lambda/\pi NA$). The UV light power and chopping frequency were optimized for detection sensitivity. The output from a laser diode (LD) (TOLD 9201, Toshiba) was collimated by a lens with $f = 5$ mm (Spindler & Hoyer) and, subsequently focused and divided into two beams of similar intensity by a custom-made hologram [17]. One of the beams, the probing beam, crossed the capillary, whereas the other beam, the reference beam, propagated through the capillary wall. The superposition of both beams yielded a fringe pattern moving according to the absorbance induced temperature of the probing zone. The TOA signal was retrieved by fringe shift detection [17]. Since acetone gives a good TOA signal, a 1% aqueous solution was used to optimize the position of the beams and the capillary to get the highest signal output. The background absorbance of the fused silica was at least an order of magnitude below buffer absorbance and did not interfere with the TOA detection.

To make a detection window in the fused-silica capillary, a small portion of the polyimide coating was removed. The detection cell was constructed by fixing the capillary (Polymicro Technologies, Phoenix, AZ, USA) between two glass plates using a transparent refractive index matching glue (Norland Products, New Brunswick, NJ, USA) as previously described [17]. The LD, the optic elements, and the detection cell were rigidly mounted into an aluminum block and thermally stabilized using a Peltier/thermistor system (Peltier element from Melcor, Trenton, NJ, USA, and controller Model LDT-5412 from ILX Lightwave, Bozeman, MT, USA). A photodiode array with eight elements (KOM 2045, Siemens, Germany) was connected to a divider circuit (Model 301-DIV, UDT, Hawthorne, CA, USA) rendering a position-sensitive signal output to measure the displacement of the interference fringes. The displacement is proportional to the RI change (Δn) within the detection volume and therefore also to the sample absorbance at the pumping wavelength. The Δn signal was fed into a lock-in amplifier (SR 530, Standford

Research Systems) to be demodulated and amplified. The demodulated signal was acquired by a Macintosh IIfx computer using the LabVIEW hard- and software package (National Instruments, Austin, TX, USA) and processed by the Igor software (WaveMetrix, Lake Oswego, OR, USA).

2.2. Capillary electrophoresis

Most separations were performed with hydrostatic injection (by manually rising the injection vial) using a laboratory-built CE system consisting of a high-voltage power supply (Model HCN 14-20 000, FUG Elektronik, Rosenheim, Germany) and fused-silica capillaries of 10 and 20 μm I.D. For some separations an automated injection system (Prince; Lauer Labs., Emmen, Netherlands) was used for pressure induced injection. The injection parameters (height/pressure and time) and the separation voltages were optimized with respect to band broadening and detection sensitivity. The capillaries were initially rinsed with a 1 *M* NaOH solution for 20 min and before each run with 0.1 *M* NaOH.

The protein solutions (see Table 1) were prepared each day in HPLC-grade water. 2-(N-Cyclohexylamino)ethanesulphonic acid (CHES, 20 m*M*) or 20 m*M* 3-cyclohexylamino-1-propanesulphonic acid (CAPS) buffers at a pH above the isoelectric point of the proteins (i.e. pH 10–11) were used to charge the proteins negatively in order to reduce the interactions with the negatively charged inner capillary surface and thus to increase the separation efficiency. Buffer solutions were filtered (0.22 μm) and degassed in an ultrasonic bath before use.

2.3. Chemicals

Proteins were purchased from Sigma (St. Louis, MO, USA), buffers from Fluka (Switzerland), and KCl and acetone from Merck (Darmstadt, Germany).

Table 1
Limits of detection (LOD) for some proteins obtained with TOA and LIF detection

Protein	M_r	Capillary I.D. (μm)	TOA LOD		LIF Concentration LOD (*M*)	Ref.
			Concentration (*M*)	Mass (mol)		
BSA	69 000	10	$1.6 \cdot 10^{-7}$	$1.7 \cdot 10^{-17}$		This work
		20	$7 \cdot 10^{-9}$	$5.5 \cdot 10^{-17}$		This work
		50			$2 \cdot 10^{-10}$	[3][a]
		75			$4 \cdot 10^{-9}$	[8][b]
α-Lactalbumin	14 000	10	$2.5 \cdot 10^{-6}$	$2.8 \cdot 10^{-16}$		This work
β-Lactoglobulin A	17 500	10	$3.1 \cdot 10^{-6}$	$3.3 \cdot 10^{-16}$		This work
β-Lactoglobulin B	17 500	10	$2.6 \cdot 10^{-6}$	$2.8 \cdot 10^{-16}$		This work
Carbonic anhydrase	31 000	20	$7.0 \cdot 10^{-8}$	$2.2 \cdot 10^{-16}$		This work
Peroxidase	40 000	20	$7.6 \cdot 10^{-7}$	$1.2 \cdot 10^{-15}$		This work
Pepsin	33 000	20	$7.6 \cdot 10^{-7}$	$1.0 \cdot 10^{-14}$		This work
Myoglobin	17 500	20	$4.2 \cdot 10^{-8}$	$1.3 \cdot 10^{-16}$		This work
Alkaline phosphatase	140 000	20	$1.8 \cdot 10^{-7}$	$5.6 \cdot 10^{-16}$		This work
Trypsin inhibitor	7 500	20	$1.8 \cdot 10^{-5}$	$1.4 \cdot 10^{-14}$		This work
Catalase	240 000	20	$2.8 \cdot 10^{-7}$	$2.2 \cdot 10^{-16}$		This work
Conalbumin	77 000	25			$2.5 \cdot 10^{-8}$	[7]
		50			$1.0 \cdot 10^{-10}$	[3][a]
		75			$1.3 \cdot 10^{-9}$	[8][b]

[a] A Ar^+ laser lasing at 275 nm was used.
[b] A pulsed excimer laser lasing at 248 nm was used.

3. Results and discussion

3.1. Sensitivity optimization

The TOA detection noise is mainly due to background absorption by buffer impurities and, to a lesser extent, due to the lock-in amplifier, the signal conditioner, the wavelength stability of the probe laser, the power of the pump lasers and, to the precision in the data acquisition [15]. BSA was used to maximize the TOA signal-to-noise (S/N) ratio with respect to these parameters.

The dependence of the S/N ratio with pumping power is shown in Fig. 2. S/N increases up to 20 mW where it remains approximately constant up to 50 mW. This effect is most likely due to a saturation in the UV absorbance of the sample. An illumination power of 50 mW (i.e., an intensity density of ca. 1 MW/cm^2) corresponds to $6.5 \cdot 10^{16}$ photons per second shared by only ca. $2.6 \cdot 10^6$ molecules of BSA (pumping spot diameter, 2.6 μm; path length, 10 μm) yielding for a molecule, on the average, one excitation every 40 ps, which should suffice to saturate the UV absorption of the protein having an excited state lifetime in the low nanosecond range. In addition, high pumping powers are not always desired because it could lead to photo-bleaching [15] and cell degradation [16]. Consequently, 20 mW was used to pump the sample in the separation experiments reported here.

The best S/N conditions, within the expected chopper frequency range of 30 to 800 Hz, are found near 300 Hz. To prevent aliasing with the mains frequency, an odd chopper frequency of 314 Hz was thus selected for all experiments.

As the lasing wavelength of the LD jitters under some operating conditions, quiet operation required a careful adjustment of their drive currents and operating temperatures.

Several separations of the BSA standard sample were performed under equal CE conditions but various separation voltages. The corresponding S/N ratios and peak efficiencies are shown in Fig. 3. The decrease in the S/N with increasing applied voltage observed is most likely related to the increased migration velocity of the analytes leading to shorter peaks (transition times in the detector), which, in combination with the relatively long lock-in time constants, leads to decreased signal levels. Besides, higher flow-rates remove the produced heat more efficiently from the detection region and might lead

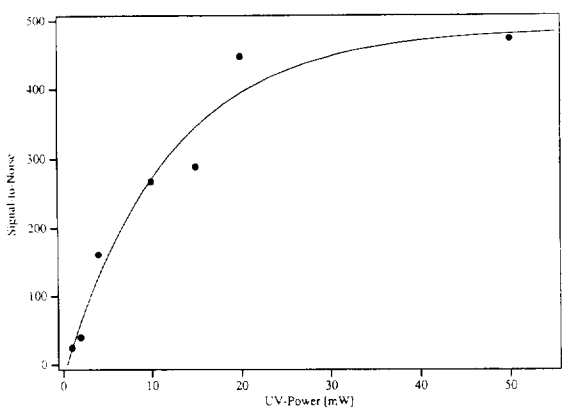

Fig. 2. Dependence of the S/N ratio with the pumping power under CE conditions. Separation voltage, 5 kV; chopper frequency, 314 Hz; sample, BSA (5.5 mg/ml in water); buffer, 20 mM CHES at pH 10.2; capillary 19 cm (14.5 cm to detector) × 10 μm I.D., injection by gravity, 7 cm/120 s; lock-in detection sensitivity, 100 mV; time constant, 0.3 s. The curve is an exponential fit.

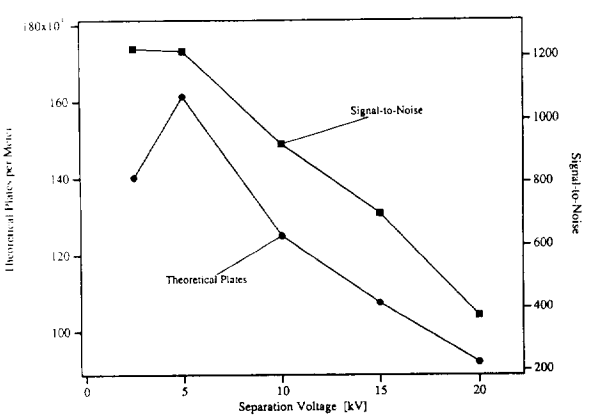

Fig. 3. Variation of the S/N ratio and peak efficiency with the separation voltage. The capillary length is 19 cm therefore at 20 kV the electical field is 1053 V/cm. ● = Theoretical plates; ■ = S/N. Separation conditions as in Fig. 2.

to reduced TOA signals at higher electrical fields.

Theory [18] predicts an increase in efficiency at higher applied voltages if the peak broadening is entirely dominated by diffusion. However, as can be seen in Fig. 3, where the efficiency as a function of applied voltage is plotted, this is not the case here. The reason for this anomalous behaviour could be attributed to the instrumental limitations of the data acquisition and/or Joule heating. The sampling rate of only 5 Hz, in combination with the relatively long time constant used might have resulted in a peak broadening. In this case, the shorter the FWHM (full width at half maximum) duration of the peak (i.e. at higher applied voltages), the more pronounced would be the observed artifact. Adsorption at the capillary wall, which is expected to becomes more apparent at higher applied voltages, is not expected to contribute to the observed peak broadening because BSA is negatively charged at basic pH.

To characterize the instrument the response factor can be defined as

$$R_{TOA} = \frac{dU_{TOA}}{dA} \quad (1)$$

where R_{TOA} is the response, dU is the change of the signal output voltage to be recorded and dA the change in absorbance within the detection lumen. R_{TOA} for our device is given by [15]:

$$R_{TOA} = \frac{4.606KH}{\pi S} \cdot \frac{CP_{UV}}{\lambda_{probe}\sigma} \cdot \frac{dn}{dT} \cdot \left(1 - \frac{I.D.}{O.D.}\right) \quad (2)$$

where K is the amplification constant of the photodiode array conditioner (K = 10 V), H the efficiency of the lock-in amplifier which is determined empirically (H = ca. 4 V), S the sensitivity setting in the lock-in amplifier (S was 50 or 100 mV), C the fringe contrast (C = ca. 0.65), P_{UV} the power of the Ar^+ laser, λ_{probe} the wavelength of the LD (ca. 672 nm), σ = 1.38 W K^{-1} m^{-1} is the thermal conductivity of fused silica, dn/dT the temperature coefficient of the RI of the buffer (value for water $1.07 \cdot 10^{-4}$ K^{-1}), I.D. (10 or 20 μm) and O.D. (340 μm) the internal and external capillary diameters, respectively. For capillaries whose I.D./O.D. $\ll 1$ (in the present work this value was ca. $3 \cdot 10^{-2}$), the TOA response does not depend on the capillary I.D.. As the absorbance, however, is increasing with I.D., the detector output, U_{TOA}, is increasing accordingly and therefore higher signals are expected for larger capillary I.D.s. Further, as Eq. 2 shows, the TOA signal can be enhanced by increasing the UV pumping power as shown in Fig. 2.

3.2. CE separations and LODs

Fig. 4 shows the electropherogram of peroxidase, carbonic anhydrase, myoglobin and alkaline phosphatase separated in a 20-μm capillary and Fig. 5 displays the separation of BSA, α-lactalbumin and β-lactoglobulins A and B in 10 (a) and 20 μm I.D. (b) capillaries. The high electric field of 500 V/cm applied during the separation, in conjunction with the short capillary (14.5 cm to detector) for the electropherogram shown in Fig. 5a resulted in a separation time of only 2 min at an efficiency (for BSA) of ca. 60 000 theoretical plates. The good heat dissipation provided by the 10 μm I.D. × 350

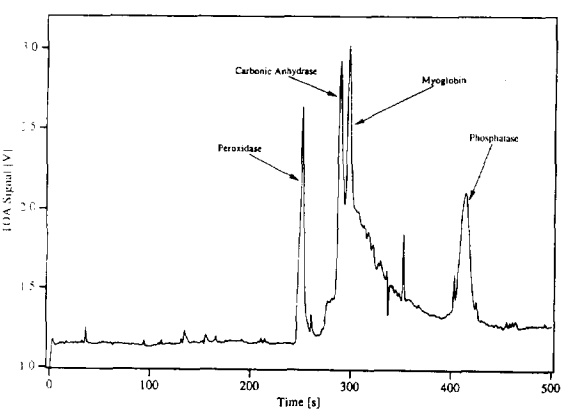

Fig. 4. CE separation of peroxidase (0.7 mg/ml), carbonic anhydrase (0.7 mg/ml), myoglobin (0.14 mg/ml) and alkaline phosphatase (3.5 mg/ml) detected by TOA. Buffer, 20 mM CAPS at pH 11; pumping power, 10 mW; capillary, 75 cm (50 cm to detector) × 20 μm I.D., hydrodynamic injection, 50 mb/30 s; voltage, 30 kV; current, 6 μA; lock-in sensitivity, 5 mV.

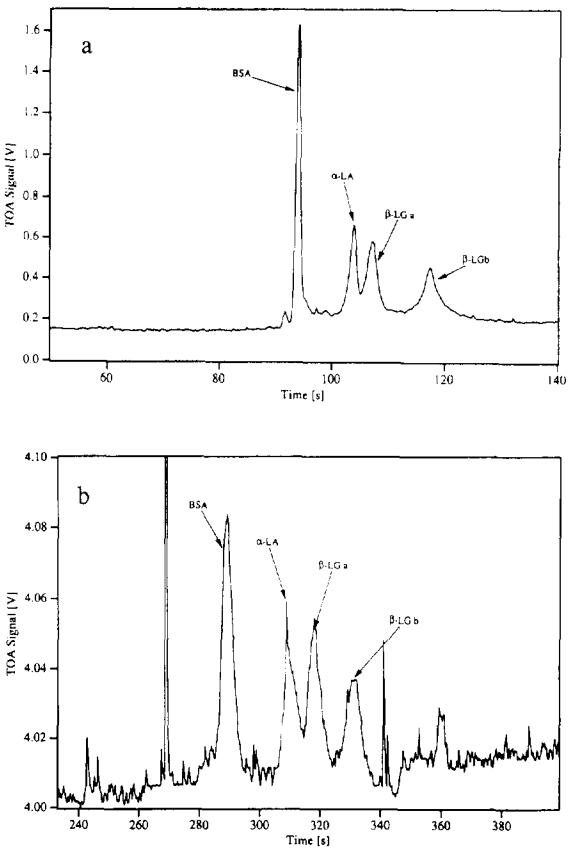

Fig. 5. CE separation of four whey proteins using TOA detection. Sample: BSA, α-lactalbumin (α-LA), β-lactoglobulin A (β-LG a), β-lactoglobulin B (β-LGb). Conditions for (a): sample concentration, 1 mg/ml of each in water; buffer, 20 m*M* CHES at pH 10.2; pumping power, 20 mW; capillary, 19 cm (14.5 cm to detector) × 10 μm I.D.; injection, 7 cm/90 s; voltage, 10 kV, current, 1 μA; lock-in detection sensitivity, 100 mV. Conditions for (b): sample concentration, 0.01 mg/ml; buffer, 20 m*M* CAPS + 20 m*M* KCl at pH 11; capillary, 75 cm (50 cm to detector) × 20 μm I.D.; injection, 300 mbar/30 s; voltage, 30 kV, current, 7 μA; lock-in detection sensitivity, 50 mV.

μm O.D. capillaries employed allowed the increase in the separation electrical field up to 1053 V/cm resulting in fast separations without a substantial degradation in the separation efficiency. The good efficiencies obtained (ca. $3 \cdot 10^5$ to $4 \cdot 10^5$ plates/m) under the conditions used in Fig. 5a compares well with the best values reported for the proteins in question. However, these values are one order of magnitude lower than the $2 \cdot 10^6$ plates/m expected for such proteins by CE [19]. Values close to these theoretical ones have only been achieved for acidic proteins in hydrophilic coated capillaries [20].

The sensitivity of the TOA device was studied under CE conditions using diluted BSA samples at concentrations close to the LOD. The obtained LOD for 10 μm I.D. capillaries was $1.6 \cdot 10^{-7}$ *M* at $S/N = 2$ whereas those measured in 20 μm capillaries (Fig. 5b) was $1.5 \cdot 10^{-8}$ *M* (the measuring conditions where not exactly identical).

As it was observed that a He–Ne probe laser results in lower noise in RI detection [15], the LD was replaced by a He–Ne laser to improve the LODs. Fig. 5b shows the separation of a solution containing $1.4 \cdot 10^{-7}$ *M* BSA within a 20 μm tube. When the hydrodynamic injection is increased to 300 mbar/30 s a LOD of $1.5 \cdot 10^{-8}$ *M* was obtained (Fig. 5b). Beyond this injection condition (e.g. 500 mbar/30 s) the baseline resolution is lost and a LOD of $7 \cdot 10^{-9}$ *M* corresponding to a mass detection limit for BSA of $5.5 \cdot 10^{-17}$ mol injected. Since the beam diameter of the probe laser is ca. 2.6 μm, the mass in the pumping volume, which contributes to the TOA signal, is less than $7.4 \cdot 10^{-22}$ mol or less than 450 BSA molecules. The LODs for all proteins studied are summarized in Table 1. Table 1 also includes the most sensitive results obtained with native LIF techniques. The LOD obtained with native LIF detection for conalbumin [7] of $2.5 \cdot 10^{-8}$ *M* is slightly larger than the one of $7 \cdot 10^{-9}$ *M* we obtained for BSA with TOA at the same wavelength and a similar I.D. capillary (I.D. = 25 and 20 μm in the LIF and TOA detection, respectively).

4. Conclusions

It is shown here that the sensitivity delivered by TOA detection in CE is comparable to that obtained with LIF detection for proteins displaying native fluorescence under similar conditions. Since all proteins absorb UV light and, only a few of them present native fluorescence, the here reported LODs using TOA detection for the

other proteins displaying weak fluorescence most likely could not be matched by LIF. Besides, and considering that when using TOA derivatizations are not needed, it has a larger field of applications than LIF. Higher TOA sensitivities could be gained using flared capillaries [21] having e.g. 25 μm in the separation section and 75 μm at the bubble.

Acknowledgements

J.M.S. would like to thank to the University of Alcalá de Henares and Comunidad Autónoma de Madrid (Spain) for their financial support. J.C.D.-M. acknowledges to DGICYT the grant for the project PB91-0077C0302. This project was supported by the CERS (Commission pour l'Encouragement de la Recherche Scientifique, Switzerland, project 2156.1). We thank G. Bruin and E. Gassmann for reviewing the manuscript.

References

[1] S. Mäkinen-Kiljunen and T. Palosuo, *Allergy*, 47, (1992) 347.
[2] *European Pharmacopoeia, Human Insulin Monograph*, 1993.
[3] T.T. Lee and E.S. Yeung, *J. Chromatogr.*, 595 (1992) 319–325.
[4] R.L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 489A.
[5] A.M. Hoyt, S.C. Beale, J.P. Lermann and J.W. Jorgenson, *J. Microcol. Sep.*, 5 (1993) 325.
[6] F. Foret, E. Szoko and B.L. Karger, *J. Chromatogr.* 608 (1992) 3.
[7] D.F. Swaile and M.J. Sepaniak, *J. Liq. Chromatogr.*, 14 (1991) 869-893.
[8] K.C. Chang, G.M. Janini, G.M. Muschik and H.J. Issaq, *J. Liq. Chromatogr.*, 16 (1993) 1877.
[9] B. Nickerson and J.W. Jorgenson, *J. Chromatogr.*, 480 (1989) 157.
[10] N.J. Dovichi, *CRC Crit. Rev. Anal. Chem.*, 17 (1987) 357–423.
[11] E.S. Yeung, *Detectors for Liquid Chromatography*, Wiley, New York, 1986.
[12] L.H. Fang and R.L. Swofford, in D.S. Klinger (Editor), *Ultrasensitive Laser Spectroscopy*, Academic Press, New York, 1983.
[13] A.E. Bruno, A. Paulus and D.J. Bornhop, *Appl. Spectrosc.*, 45 (1991) 462.
[14] J.M. Saz and J.C. Díez-Masa, *J. Liq. Chromatogr.*, 17 (1994) 499–520.
[15] B. Krattiger, A.E. Bruno, H.M. Widmer and R. Dändliker, *Anal. Chem.*, in press.
[16] K.C. Waldron and N.J. Dovichi, *Anal. Chem.*, 64 (1992) 1396.
[17] B. Krattiger, G.J.M. Bruin and A.E. Bruno, *Anal. Chem.*, 66 (1994) 1.
[18] S.F.Y. Li, *Capillary Electrophoresis*, Elsevier, Amsterdam, 1992.
[19] A. Cifuentes, J.M. Santos, M. Frutos and J.C. Díez-Masa, *J. Chromatogr. A*, 652 (1993) 161.
[20] D. Schalmalzing, C.A. Pigee, F. Foret, E. Carrilho and B.L. Karger, *J. Chromatogr. A*, 652 (1993) 149.
[21] J.M. Saz, B. Krattiger and A.E. Bruno, *Anal. Methods Instrum.*, 4 (1993) 203.