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Fiber optic modified thermal lens detector system for the determination of amino acids

B.S. Seidel*, W. Faubel

Forschungszentrum Karlsruhe, Institut für Instrumentelle Analytik, Postfach 3640, 76021 Karlsruhe, Germany

Abstract

A novel fiber optic modified thermal lens detector in combination with capillary electrophoresis (CE) is described. The probe and pump lasers used in thermal lensing were adapted via optical fibers to a commercial injection and high voltage CE system. The detector performance is demonstrated by monitoring mixtures of derivatised amino acids. Amino acids are labeled with 4-dimethylazobenzene-4-sulfonyl-chloride as absorbance reagent. Therefore all amino acids possess a large molar absorptivity in the visible region which because of its stability can be qualitatively and quantitatively detected with only one excitation line. The miniaturization of the thermal lens detector and the separation of the excitation and probe laser from the sensor head with optical waveguides permits combination with typical laboratory CE systems, which generally allow treatment of microliter volumes of amino acids.

Keywords: Detection, CE; Fiber optics thermal lens detection; Instrumentation; Thermal lens detection; Amino acids

1. Introduction

Amino acids are one of the most important classes in biological chemistry. The determination of minute quantities of amino acids in microliter volumes allows the characterization of peptides, DNA sequencing in singular cells and protein sequencing with classical Edman degradation [1]. In general, mixtures of amino acids are qualitatively determined with a chromatographic separation technique, whereas the detection is mostly done spectroscopically. Sensitive detection techniques using micron-sized capillaries are frequently based on laser spectrometric methods [2]. Because amino acids do not have a natural absorbance in the UV and visible spectral range, labeling of the molecules is necessary when using optical detection techniques. One commonoly used optical method is laser fluorescence spectrometry, for which the limits of detection (LOD) in the subattomole range have been reported [3,4]. Another method is the labeling with chromophoric reagents and usual UV–vis absorbance detection [5]. Phenyl isothiocyanate is well known as reagent in the Edman degradation. The detection of the derivatized reagent is at 254 nm, which is unusual in laser spectroscopy. Lin and Chang [6] used 4-dimethylazobenzene-4-sulfonyl (dabsyl) chloride, for qualitative and quantitative analyses of amino acids, which creates red colored dabsyl derivatives which could be visualized on thin-layer plates. Later it was also used with HPLC [7]. The application of photothermal spectroscopy with capillary electrophoresis (CE) for the separation of amino acids [3,8] or nucleotides [9] is mainly used with derivatization. Indirect detection methods, which use a background absorber in the buffer system [10,11], broaden the application of this detection method, but suffer from the limitations of buffer contaminants.

*Corresponding author.

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The principal idea of thermal lensing is simple: an excitation light beam passes through a sample of interest, the light is tuned to an absorption line of the analyte, and the optical energy is absorbed by the medium [12]. The molecules are excited into vibrational, rotational or electronic states by absorption of light. The excited molecules release the excitation energy in form of heat through nonradiative relaxation processes. This heating of the medium causes a change of the refractive index. This effect can be monitored as a change in the probe beam intensity passing through a pinhole. The thermal lens signal on the photodiode depends on the refractive index profile, induced in the sample by the pump beam. Under equilibrium conditions, the temperature change is related to the refractive index through the differential equation:

$$dn/dT = (dn/dT)_\rho + (dn/d\rho)(d\rho/dT)_\rho \quad (1)$$

where n is the refractive index, T the temperature and ρ the density of the sample. The temperature coefficient of the index of refraction, dn/dT is determined primarily by changes in the sample density (second term). Most liquids expand when heated, resulting in a negative value of dn/dT.

Photothermal methods reach the required detection limits without chemical enrichment steps. However, they still have the severe limitation that many time consuming steps are needed to align the laser beams, requiring highly skilled staff. For the solution of this problem, in our laboratory miniaturized photothermal systems have been developed [12].

In this paper, CE with an integrated miniaturized fiber optic thermal lens detector for determination of dabsylated amino acids is described. The addition of electroosmotic flow modifier improves the separation efficiency and the signal-to-noise ratio of the detector head.

2. Experimental

2.1. Apparatus

The thermal lens detector is shown in Fig. 1. It is the newest fiber optic version of the improved detector system, which we have mentioned before

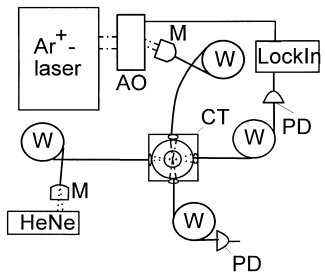

Fig. 1. Thermal lens schematic view. Pump beam is a 150 mW Ar^+ laser, whose beam is modulated with an acoustooptical modulator (80 Hz). The probe beam is a 1 mW He–Ne laser, M = microscope objectives, PD = photodiodes, CT = capillary stage and W = optical fibers.

[13,14]. A 1 mW He–Ne laser (632.8 nm) from Uniphase (Eching, Germany) is focused with a 40× microscope objective (Spindler and Hoyer, Göttingen, Germany) inside a monomode optical fiber. The light is guided to the capillary stage and is focused with a 12-mm achromate lens (Spindler&Hoyer) inside the capillary. After crossing the capillary window, the beam propagates to a 25-μm multimode fiber (BTO Bungert, Weil der Stadt, Germany) instead of a pinhole, which collects and transports the light to a remote detection position. The intensity of the so called probe beam is measured with a photodiode, (S1336-BQ, Hamamatsu, Herrsching, Germany). An Ar ion laser (Innova 200, Coherent, Dieburg, Germany) is used as pump beam. This laser system delivers up to 20 W continuous wave light at a wavelength of $\lambda = 488$ nm, and 570 mW at $\lambda = 458$ nm. The wavelength of 458 nm is suitable for the detection of the dabsylated amino acids in aqueous solutions. The pump beam is intensity modulated by a chopper or an acoustooptical modulator (A.A. Sa Opto-Electronique, Versaille, France), which gives simultaneous the reference signal of the lockin amplifier to permit phase sensitive detection. The beam guided by a 25× microscope objective (Spindler&Hoyer) into a 50-μm multimode optical fiber (BTO Bungert) is focused inside the capillary and crosses the probe beam at right angles. The pump beam changes the refractive index of the flowing medium and deflects the probe beam and causes an intensity change, which is detected as thermal lens (TL) signal. The TL signal is preamplified and fed to a lockin amplifier and the data

are recorded with a personal computer. Another photodiode records the intensity of the pump beam and is used for standardization. The distance between the collecting fiber and the thermal lens excitation region is only 250 μm. As earlier reported [12] this small distance allows highly sensitive detection in the near field regime, and thus an integration of miniaturized detector head in CE systems is possible.

The CE system consist of the high voltage power supply and the modular injector (Spectra Phoresis 100, ThermoQuest, Egelsbach, Germany), the electropherograms were recorded with the Gynkosoft software (Gynkothek, Munich, Germany). A 55 cm (35 cm effective length)×50 μm I.D. fused-silica fiber was used for all separations.

2.2. Reagents and samples

All reagents were of analytical certified grade and solutions were prepared by dissolution in distilled and deionized water. The buffer system consisting of 100 m*M* $NaBO_3$–NaOH, pH 9.2 was purchased from Merck (Darmstadt, Germany). A 50 m*M* sodium dodecyl sulfate (SDS) (Fluka, Buchs, Swiss) solution was used as micellar reagent. Methanol (15%) and tetrahydrofuran (THF) (1%) (Merck) was added to the solvent to improve the separation efficiency.

Each amino acid (Fluka) (500 μ*M*) was dissolved in sodium carbonate–sodiumhydrogen carbonate buffer, pH 9.0. The pH was fixed at 9.0, because N-dabsyl-acid with its maximum absorbance at 450 nm is favorably formed [4].

Dabsyl chloride (Fluka) (1 m*M*) was dissolved in acetone and added to each amino acid. The mixture was tightly stoppered and heated to 70°C in a water bath for 5 min. After reaction the color of the solution turned from red to orange and samples were ready for injection into the CE system.

3. Results and discussion

The separation time of six amino acids was optimized using micellar electrokinetic chromatography as separation technique. With addition of 15% methanol and 1% THF, the retention times were shortened by a factor of 3. Because nonaqueous medium possess a larger dn/dT value (Eq. (1)) the signal-to-noise ratio improved. In Fig. 2, the CE separation of six amino acids is shown. All six amino acids are well separated in a separation time between 6 and 11 min. The diagram also shows some minor peaks, which have to be assigned to the absorbance reagent.

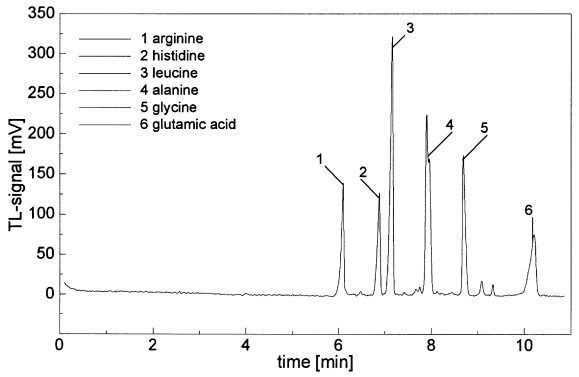

Fig. 2. CE separation, buffer (100 m*M* $NaBO_3$–NaOH, 50 m*M* SDS with 15% methanol and 1% THF); pH 9.3; voltage: 25 kV, capillary = 55 cm (35 cm effective length)×50 μm I.D.; TL detector, 458 nm; 150 mW; 80 Hz.

The concentration of each amino acid was between $3 \cdot 10^{-6}$ and $5 \cdot 10^{-6}$ *M*. In order to avoid absorbance peaks resulting from the reagent, the ratio of 1:1 was chosen for the amino acid to the absorbance reagent.

The calibration curve (Fig. 3) for the glycine derivative was linear ($r = 0.998$) over more than two orders of magnitude. The LOD with a signal-to-noise ratio of 3:1 was about $1.8 \cdot 10^{-7}$ *M*. Assuming a detection volume of 50 pl we can calculate a

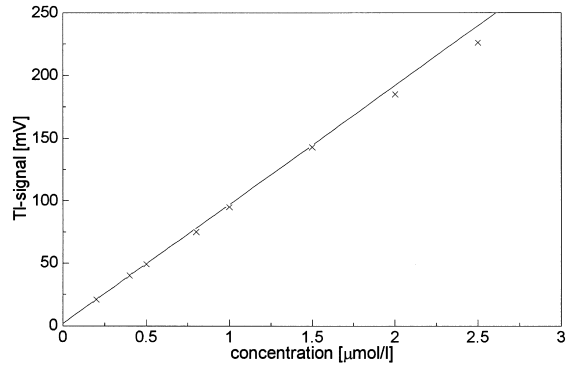

Fig. 3. Calibration curve of the glycine derivative, after derivation with 4-dimethylaminoazobenzene-4-sulfonyl chloride.

detection limit of 10 amol. This compares very well to photothermal results of 200 amol for dabsyl chloride reported by Yu and Dovichi [15] and 10 amol for the dansyl derivative reported by Yeung [10]. Dovichi reported a limit of detection of 0.009 amol for fluorescein isothiocyanate derivatives [3].

4. Conclusions

At present, there is a large interest in sequence determination of minute quantities of proteins. The thermal lens detector system in combination with CE handles smallest amounts of samples. Because the cross beam setup is independent of the optical pathlength inside the capillary, the smallest amounts of amino acids can be detected. The great potential of the photothermal technique, as a routine method for CE, lies in the miniaturization of the system. With the development of the optical fiber based miniaturized photothermal detector head it is possible to avoid time consuming and highly sophisticated adjustment procedures and to separate the optoelectronic devices from the capillary stage.

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