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Journal of Chromatography A, 930 (2001) 165–169

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Consideration on peak shape in a batch-type chemiluminescence detection cell for capillary electrophoresis

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Received 15 March 2001; received in revised form 25 July 2001; accepted 31 July 2001

Abstract

Peak areas, peak heights, and apparent theoretical plate numbers were examined as a function of sample injection times by use of the batch-type CL detection cell. Comparing the experimental data with those obtained by absorption detector, some considerations were carried out about the peak shape. The peak shape in CL detection was influenced by not only concentration distribution of sample in a sample zone but also sample diffusion and CL reaction at the capillary outlet. The sample injection time of ca. 35 s was recommended for the present CE with CL detector. The injection time much influenced peak shape as well as sensitivity in the CL detection cell.

Keywords: Peak shape; Chemiluminescence detection; Detection, CE

1. Introduction

Several groups have studied on capillary electrophoresis (CE) with chemiluminescence (CL) detector [1–3]. The new system can offer an excellent analytical selectivity and sensitivity [4]. Absorption and fluorescence detectors have been most commonly used for CE, which are performed with on-capillary detection. On the other hand, CL detection requires post column reaction at a tip of capillary. We have developed several CL detection cells for CE [5–11]. They were roughly divided into two types, flow- [5–8] and batch-types [9–11]. In the flow-type a CL reagent solution was delivered through a Teflon-tube by a pump. An eluant from the capillary was mixed at a tip of capillary with the CL reagent solution. The flow-type cell possessed some merits in the CE with CL detector. However, the whole system using the flow-type cell became complicated. And it was difficult and troublesome to operate the system. We have, then, proposed and developed the batch-type CL detection cell for CE. A CL reagent solution was there just put in the batch-type cell. The system did not need any complex construction and expensive implements. An eluant from the capillary was mixed with the CL reagent solution which was not flowing in the batch-type cell. We had been anxious about that, if the CL reaction rate is not so high, the reaction might lead to peak broadening. However, we could have satisfactory results in the peak shapes as described in the previous papers [9–11], the CL reaction must be almost instantaneous. The typical electropherogram is shown in Fig. 1B) and the peak

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)01170-0

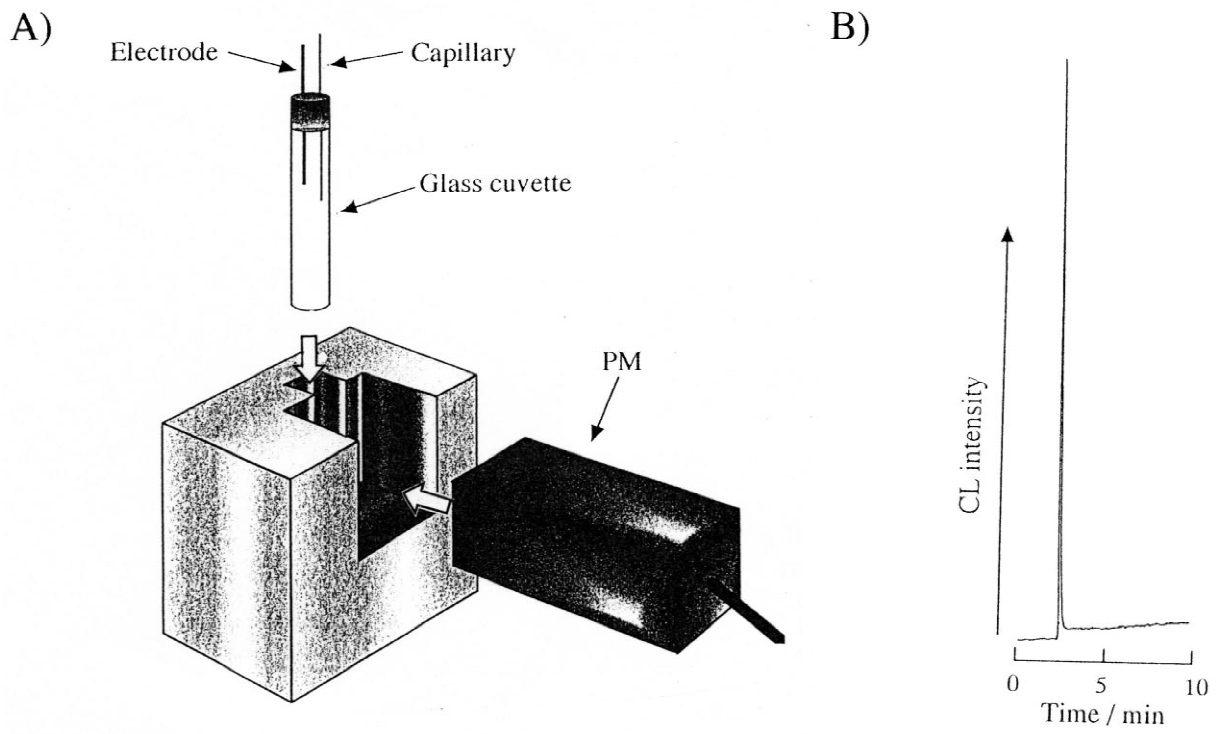

Fig. 1. (A) Schematic diagram of batch-type CL detection cell of a glass cuvette and (B) typical electropherogram of Dns–Trp (1×10^{-5} *M*).

height did not change until 7th repeated injection [11].

In this study, we examined peak areas, peak heights, and apparent theoretical plate numbers as a function of sample injection times, by use of the batch-type CL detection cell. Comparing the experimental data with those obtained by absorption detector, we made consideration on peak shape in the batch-type CL detection cell for CE. Although the obtained data here might be fragmentary, they provided a clue for understanding the effect of CL on detection property in CE.

2. Experimental

2.1. Reagents

All of the reagents used were of commercially available special grade. Ion exchanged water was distilled for use. L-Tryptophan labeled with (5-[dimethylamino]naphthalene-1-sulfonyl chloride) (Dns–Trp) was purchased from Sigma Chemical Co. Bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl]oxalate (TDPO) was received from Wako Pure Chemical Industries, Ltd.

2.2. CL detection cell [11]

Schematic diagrams of the batch-type CL detection cell of a glass cuvette is shown in Fig. 1A). The cell had an inner diameter of 5 mm and the inner volume of about 0.7 ml. A capillary and a platinum wire as a grounding electrode were inserted into the cell through the upper silicon rubber. The detection cell also worked as an outlet reservoir. The cell was put just in front of photosensor modiur (PM).

2.3. CE with CL detection [11]

A high voltage (15 kV) was applied to electrodes using a DC power supplier (Model HCZE-30PNO. 25, Matsusada Precision Devices). A fused-silica capillary of 40 cm length $\times$50 μm I.D. (GL Sci-

ences) was used. An aliquot of mixture of 2 m*M* TDPO acetonitrile solution (40 ml) and 30 wt% H_2O_2 solution (280 μl) was added into the cell. Dns–Trp was used here as a sample. The sample was dissolved in 0.1 *M* tris-borate buffer (pH 7.0) which was used as a migration buffer. Sample injections were performed by siphoning from a height of 15 cm. The sample was migrated in the migration buffer toward the CL detection cell and mixed with the reagent. The resulting CL at the capillary outlet was directly captured by the PM which was equipped in CL detector (Model EN-2 1, Kimoto Electric). The output from the detector was fed to an integrator (Chromatopac C-R6A, Shimadzu).

3. Results and discussion

Under ideal conditions in CE (an interaction between the inner wall of capillary and an analyte is negligible, plug flow occurs in the capillary, etc.), an expanse of sample zone in the capillary is contributed to only axial diffusion (that is, radial diffusion and convection effect are negligible) [12]. Consequently, variance of sample in the capillary is expressed by the following equation:

$$\sigma^2 = 2Dt = 2DlL/\mu V \quad (1)$$

σ^2, variance; *D*, diffusion coefficient; *t*, migration time; *l*, effective length of capillary; *L*, whole length of capillary; μ, mobility; and *V* applied voltage. Furthermore, a theoretical plate number (*N*) is estimated by use of σ [12]:

$$N = (t/\sigma)^2 = 16(t/W)^2 = 5.54\,(t/W_{0.5})^2 = 5.54\,(t\,I/A)^2 \quad (2)$$

W, peak width; $W_{0.5}$, half-peak width; *I*, peak height, and *A*, peak area.

Detectable ranges of UV and CL detections in CE were quite different; 5×10^{-5}–1×10^{-2} *M* in UV detection and 1×10^{-8}–1×10^{-5} *M* in CL detection for Dns–Trp [11]. Therefore, it was impossible to analyze the same sample by use of UV and CL detectors and to compare the obtained results each other. However, on the basis of the above generalization (variation of sample under ideal conditions in a capillary is independent of sample concentration), we carried out the following experiments.

We examined peak areas, peak heights, and apparent *N*s as a function of sample injection times by use of the batch-type CL detection cell. If the sample concentration is high, the CL reagent will be exhausted around the sample zone eluted from the capillary and hence the concentration must affect the peak shape. In this study, the detectable concentration of sample (Dns–Trp; 1×10^{-8}–1×10^{-5} *M*) was low enough for the concentration of CL reagent (TDPO; 2 m*M*); it was found from preliminary experiments that the effect of the sample concentration on the peak shape was not experimentally significant. Here, the sample concentration of 5×10^{-6} *M* Dns–Trp was used. The obtained data are shown in Fig. 2. The migration times did not change at all (ca. 2.2 min). Peak areas increased with an increasing of the injection time. Peak heights also increased with the time up to ca. 40 s, whereas above that they seemed to be saturated to a certain peak height.

Generally, an *N* can be calculated by Eq. (2) on the basis of the concept of Gauss distribution. However, since CL detection includes post column

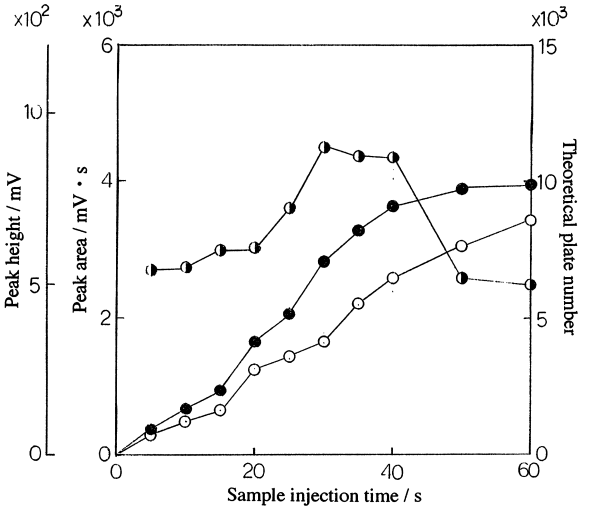

Fig. 2. Relationship between (○) peak areas, (●) peak heights, and (◑) apparent *N*s as well as sample injection times in the CE with CL detector. Conditions: Capillary, 40 cm length of 50 μm I.D.; CL reagent, 2 m*M* TDPO and 200 m*M* H_2O_2; migration buffer, 0.1 *M* tris-borate (pH 7.0); applied voltage, 15 kV; and sample, 5×10^{-6} *M* Dns–Trp.

reaction, the *N* calculated by such a way in the CE with CL detector must have a few different imports. Therefore, the *N* thus obtained was called an ''apparent'' *N* in this study. From the Eq. (2), a maximum *N* is obtained when a ratio of peak height to peak area becomes largest. In Fig. 2 the maximum value was observed at the injection time of ca. 35 s. Judging from the peak heights and the apparent *N*s in Fig. 2, the injection time of ca. 35 s was recommended for the present CE with CL detector. The peak shape in CL detection much depended on the sample diffusion at the capillary outlet which influenced CL reaction.

Fig. 3 shows peak areas, peak heights, and *N*s obtained by UV detector. UV detection (328 nm) was carried out by using a modified spectrophotometric detector (SPD-6AV, Shimadzu). A capillary of 60 cm length (40 cm effective length) and 50 μm I.D. was used. The applied voltage was 21 kV and 100 m*M* tris-borate buffer (pH 7.0) was used as a migration buffer. The migration times did not change at all (ca. 2.2 min). Peak areas increased with an increasing of the injection time. Peak heights also increased with the time up to ca. 20 s, but above that they seemed to

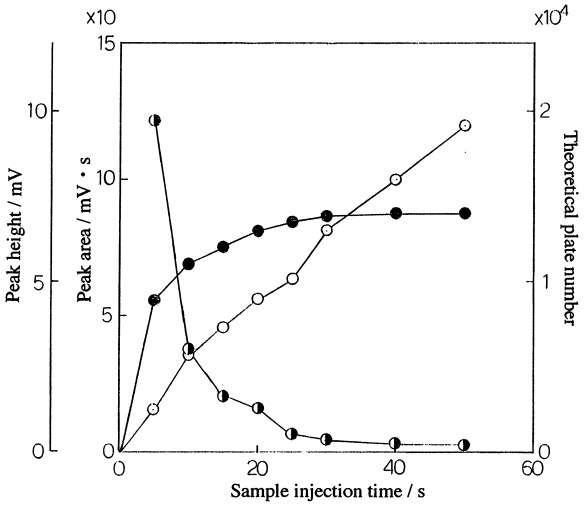

Fig. 3. Relationship between (○) peak areas, (●) peak heights, and (◑) apparent *N*s as well as sample injection times in the CE with UV detector. Conditions: Capillary, 60 cm length (effective length of 40 cm) of 50 μm I.D.; CL reagent, 2 m*M* TDPO and 200 m*M* H_2O_2; migration buffer, 0.1 *M* tris-borate (pH 7.0); applied voltage, 21 kV; detection, 328 nm; and sample, 5×10^{-3} *M* Dns–Trp.

be saturated. The beginning to the saturation of peak height in UV detection appeared earlier than that in CL detection. Consequently, quite different behavior of *N* value appeared in Fig. 3, compared with in Fig. 2. The *N*s in Fig. 3 decreased gradually with an increasing of the time. From the results in Fig. 3, the sample injection time of ca. 10 s was recommended for the CE with UV detector. The values of *N*s obtained by CL (the injection time of 35 s) and UV (the injection time of 10 s) were almost the same, and also equal to that reported in the previous work using the flow-type CL detection cell [11].

If a sample migrates in a capillary under ideal conditions, concentration distribution of sample in a sample zone should be the same for the CE systems equipped with UV and CL detectors, even though at different sample concentrations. Taking the generalization into consideration, the behaviors of the *N* values in Figs. 2 and 3 seemed to be somewhat strange at first glance. However, the difference would be easily understood by considering the followings: The peak shape in CL detection must be influenced by not only concentration distribution of sample in a sample zone but also sample diffusion and CL reaction at the capillary outlet in the batch-type cell. That is, the peak shape in the CL detector was based on the CL appearance through the post-column CL reaction at a tip of capillary which much depended on the sample diffusion in the batch-type cell.

A length of sample zone corresponding to $<$1–2% of a whole capillary length is generally recommended for CE analysis [12]. The injection time of 35 s in CL detection made the length of sample zone of 8.8 mm, which corresponded to ca. 2.2% of the whole capillary length. The injection time of 10 s in UV detection made the length of sample zone of 2.5 mm, which corresponded to ca. 0.4% of the whole capillary length. The value of 0.4% in UV detection was satisfied with the recommended condition of $<$1–2%, while 2.2% in CL detection was over the condition. The facts must be attributed to that CL detection included post column reaction at the tip of capillary, not being carried out with on-capillary. That is, as mentioned above, the peak shape in CL detection was influenced by sample diffusion in the detection cell and CL property as well as concentration distribution of sample in a sample zone.

Even the injection time of 35 s which showed the peak broadening in the UV detection indicated the best peak shape in the CL detector. As a result, the sample injection time must be examined and optimized for the CE with CL detector. The CL detection would improve not only sensitivity but also peak shape by examining the analytical conditions.

Acknowledgements

This work was supported by a grant to RCAST at Doshisha University from the Ministry of Education, Japan. This was also supported in part by Doshisha University's Research Promotion Fund.

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