Direct Observation of Capillary Electrophoretic Phenomena Using a Video Image Analysis System

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A video imaging system was developed for direct observation of the separated zones of DNS-amino acids inside a capillary during an electrophoretic run. Using a CCD video camera, the moving zone of fluorescent band was observed after irradiation of capillary with a UV lamp and monitored in 10 cm region of a capillary. On-line data acquisition in addition with the possibility of further image processing, made it possible to get a clear and real image of the separated bands of solute.

In Capillary Electrophoresis (CE), separation of few nano litre of solutes in a narrow bore tube of fused-silica can be achieved as a result of differential migration of individual solutes according to their charge to mass ratio. Using the same methodology as in micro column HPLC with minor modification, made it possible to detect a tiny amount of injected sample (ca.1-50 nL).[1] The usefulness of a video image analyzing system for the direct observation of the separation process taking place in a HPLC glass column, have been already shown in this laboratory.[2] In a similar methodology, a video imaging system was developed to investigate the zone pattern of the separated zones of solutes inside a capillary during an electrophoretic run. Precise calculation of solute's velocity, and study of the effect of loaded amount of sample on the shape of

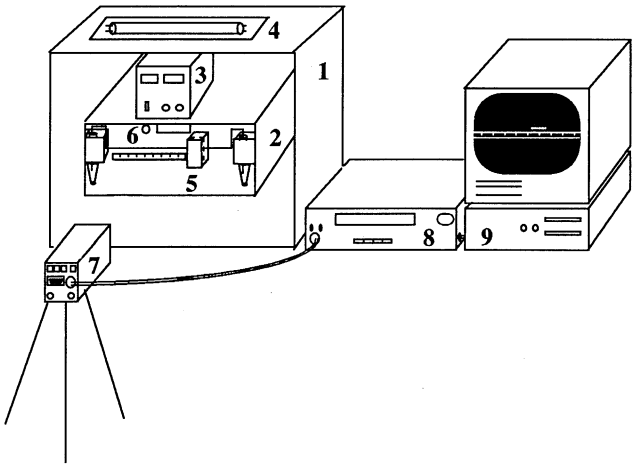

Figure 1. Developed video imaging system for the direct observation of the DNS-amino acids zone in a capillary electrophoretic run. **(1)**: Black box, **(2)**: CE compartment and UV detector, **(3)**: Power supply, **(4)**: UV lamp, **(5)**: UV cell, **(6)**: CE capillary, **(7)**: CCD camera, **(8)**: Video recorder, **(9)**: Computer and image processor.

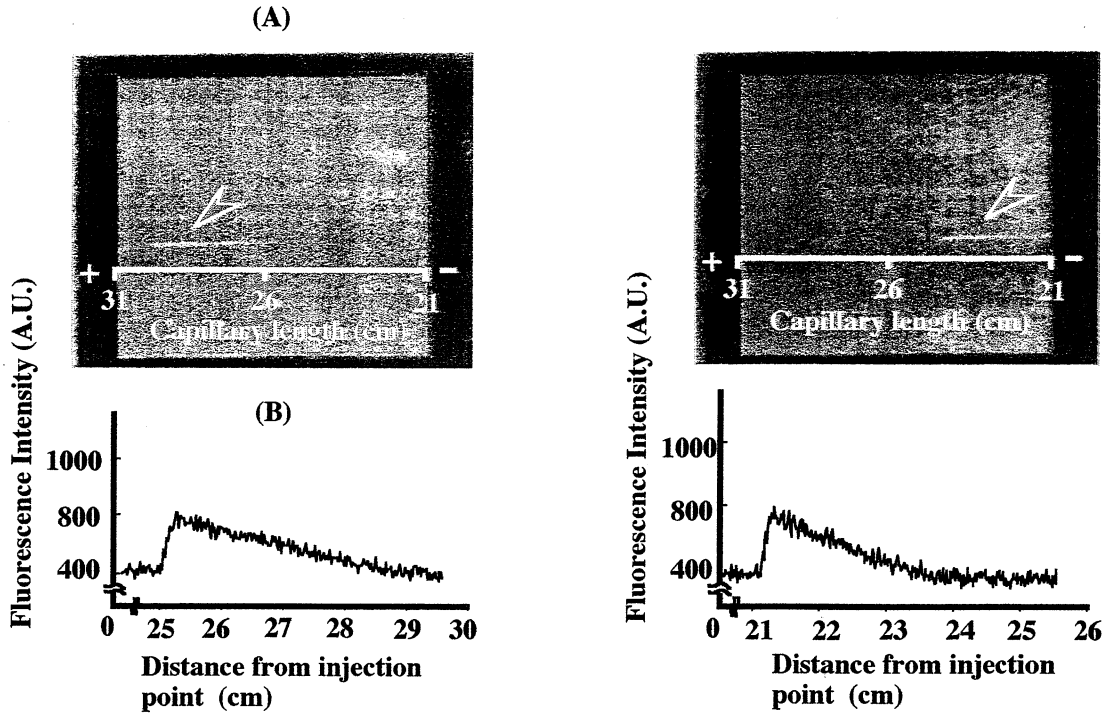

Figure 2.(A): Video images of the fluorescent zone of DNS-glycine. The sample was injected for 1.5 min. For other condition see reference and note No.4 **(B):** The result of image processing of Figure **(A)**. The data point light intensity in video images were converted to digits and the graphs were plotted using Microsoft Excel version 4.0.

zone were performed by this system. By the microscopic observation, imaging of the electrokinetic flow profiles in CE capillary have been already reported.[3] However, we aimed to visualize the separation pattern and moving zone of the solutes in a wide length of capillary during an electrophoretic run to study the insight of CE separation process.

The CE system used in this study was explained elsewhere.[4] A video camera with three charge-coupled device (DXC-930, Sony, Japan) was used as a detector to monitor the zone movement of fluorescent DNS-amino acids including glutamic acid, histidine, glycine, alanine, and asparagine (written by the order of elution). For the direct observation of solutes, polyimide coating of capillary was removed to make a 10 cm transparent window. A triacetyl cellulose sharp cut filter (SC-42, Fuji, Japan) was used to cut the excitation light provided by a UV lamp. This system was placed in a dark box as shown in Figure 1. The video images was recorded either using an standard video recorder (UVW-1400, Sony, Japan) or a time-lapsed video recorder (AG-7620A, Panasonic, Japan). The image data were analyzed using a microcomputer (Compaq, USA) equipped with an image processing board (GPB-1, Sharp, Japan). The video images was printed by a video printer (CP-11, Mitsubishi, Japan).

In Figure 2A, the real video images of DNS-glycine in two positions are shown with total exposure length of capillary to be 10 cm. The velocity was calculated by measurement of the time required for passage of the zone from one cm length of capillary (υ = 4.17 x 10^{-2} cm/s). Any behaviour of the moving zone of DNS-glycine was under direct observation. We have seen that the spatial zone length was increased in second position mainly because the capillary was overloaded by the injected amount of sample (hydrodynamic injection of 10^{-2} M (1 M = 1 mol dm^{-3}) for one min from 5 cm height). In Figure 2B, the results of image processing are shown. The peaks are suffering from tailing due to the mentioned reason. In order to study the effect of loaded amount of sample on band width, DNS-glycine with different loading times was injected into the capillary and the spatial length of the corresponding zone was measured directly from the video images. For the injection time of 15, 30, 60, and 75 sec, the spatial zone length measured at the 23th cm far from the injection point were 1, 2, 3, and 3.5 cm respectively. These observation and measurements showed the clear effect of the length of the injected amount of sample on peak broadening. Using the same method, the velocity of tested DNS-amino acids were calculated and presented in Table 1.

Using a laser as an excitation source to improve the method sensitivity, one can load the ordinary sample amounts into a capillary, consequently the peak tailing as a result of sample overloading will not occur. In that case, this system is quite versatile for the study of solute interaction with various modified capillary interior walls. In addition, interaction between drugs and proteins such as HSA or BSA as model studies can be carried out using this video imaging system, which is currently under investigation in this laboratory.

Table 1. Calculated velocity of tested DNS-amino acids using a video imaging capillary electrophoresis system

DNS-amino acid	Velocity (cm/s x 10^2)
DNS-glutamic acid	4.55
DNS-histidine	4.35
DNS-glycine	4.17
DNS-alanine	4.07
DNS-asparagine	2.70

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References and notes

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