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# Migration behavior of organic dyes based on physicochemical properties of solvents as background electrolytes in non-aqueous capillary electrophoresis

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## ABSTRACT

The migration behavior of organic fluorescent dyes (i.e., crystal violet, methyl violet base, methyl violet B base, rhodamine 6G, and rhodamine B base) in non-aqueous capillary electrophoresis (NACE) was investigated by focusing on the physicochemical properties of various organic solvents [ethanol, methanol, 2-propanol, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO)] in background electrolyte (BGE). Laser-induced fluorescence (LIF) and UV/Vis detectors were employed to observe both the migration time of organic dyes and the electroosmotic flow (EOF) in NACE, respectively. As seen in conventional aqueous BGE, the mobility of EOF in organic solvents tended to rise when the ratio between the dielectric constant and the solvent's viscosity ( $\epsilon/\eta$ ) increased in accordance with Smoluchowski's equation. However, unlike the  $\epsilon/\eta$  of pure organic solvents, the migration order of dyes changed as follows: methanol (60.0) > DMF (45.8) > ethanol (22.8) > DMSO (23.4) > 2-propanol (9.8). Since the amount of acetic acid added to balance the pH depends on the  $pK_a$  of each solvent, EOF changed when the difference in the  $\epsilon/\eta$  value was small. This resulted from the inhibition of mobility, and its difference was dependent on the  $\epsilon/\eta$  of BGEs with high ionic strength. In particular, the actual mobility of dyes in DMF showed excellent compliance with the Debye–Hückel–Onsager (DHO) theory extended by Falkenhagen and Pitts, which enabled us to analyze all dyes within 15 min with excellent resolution ( $R_s > 2.5$ ) under optimum NACE conditions (10 mM sodium borate and 4661 mM acetic acid in 100% DMF, pH 4.5). In addition, the NACE method was successfully applied for analyzing commercially available ballpoint ink pens. Thus, these results could be used to anticipate the migration order of organic dyes in a 100% NACE separation system.

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## 1. Introduction

The dye in a coloring material is capable of absorbing and reflecting a specific wavelength of visible light. Numerous types of dyes are produced in the world annually [1]. Organic fluorescent dyes have been used for coloring fabrics [2], cosmetics [3], in the production of paint pigments [4], and embedded in special solar fibers [5]. These dyes have also been used as forensic clues to identify counterfeit bills [6–8], and have become a subject of analysis in various other aspects. Organic dyes with bulky hydrocarbon rings, especially crystal violet [9], methyl violet [10], and rhodamine-

based compounds [11,12], are regarded as important in solving cases related to fiber and ink analysis in forensic science [13,14].

Thin-layer chromatography (TLC) [15], high-pressure liquid chromatography (HPLC) [16], gas chromatography-mass spectrometry (GC–MS) [17], and matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS) [18] were developed for analyzing the composition of inks to provide information about the identity and composition of questionable documents [19,20]. Each process has its own set of pros and cons. TLC is widely used for ink analysis because it is both cost-effective and produces fast analytical results; however, the method is known to be unsuitable for separating insoluble compounds [6]. GC–MS provides high S/N using thermal desorption, but absorption with polar solvents and gas flow frequently results in peak tailing [21]. MALDI-MS minimizes the process of sample preparation, but the operation system is relatively expensive compared to other lab-made equipment [18,21]. Even though capillary electrophoresis (CE) requires a well-trained

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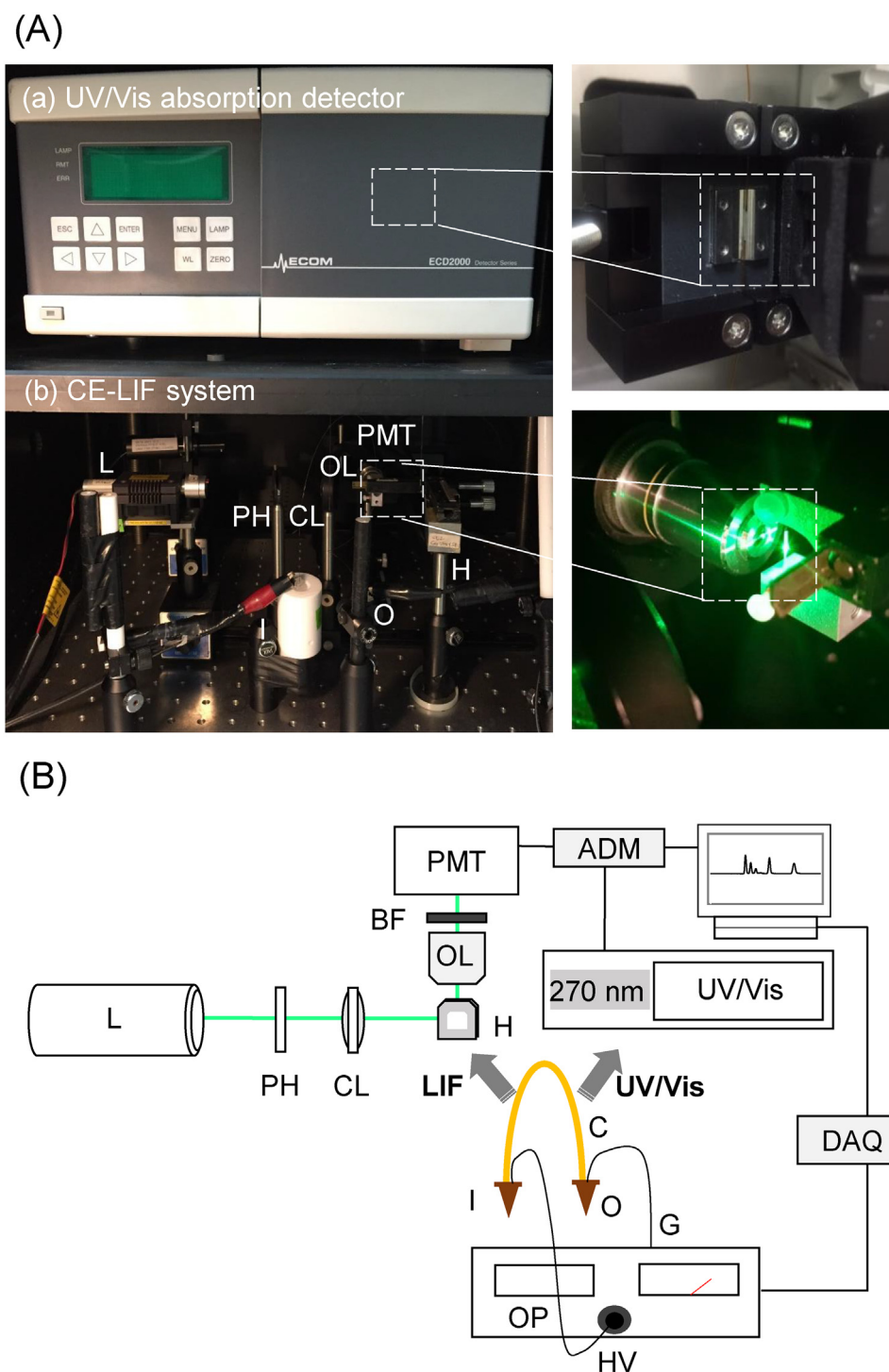
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experimenter to obtain accurate results, the method still offers many pros because it utilizes less sample for a typical analysis [6] and is cheaper to run [21]. Micellar electrokinetic chromatography and capillary zone electrophoresis are among various other CE separation techniques developed for analyzing charged or neutral compounds in inks [19,20].

Most of the synthetic organic dyes with high molecular weights are insoluble in the aqueous solvents widely used in conventional CE [22,23]. Since many ink components are influenced by pH [24],

temperature [25], humidity [26], and solvent [27], it is necessary to consider the influence of environmental factors on an analysis. Aqueous solvents in background electrolyte (BGE) generally tend to have similar  $pK_a$  values. On the other hand, the  $pK_a$  values of organic solvents tend to vary [28,29]. Having a wide selection of organic solvents with various  $pK_a$  values can be advantageous for separating numerous synthetic organic dyes [30–33]. Since most organic solvents also have dielectric constants lower than water's constant, the current in the capillary during a non-aqueous CE (NACE) exper-



**Fig. 1.** (A) Photographs of the capillary window portion aligned for (a) UV/Vis absorption and (b) CE-LIF detector. (B) Schematic diagram of full CE-LIF system with dual-detection mode. Abbreviations: L, 532 nm laser; PH, Pinhole; CL, convex lens; I, inlet; O, outlet; C, capillary; H, capillary holder; OL, objective lens; BF, Band-pass filter; PMT, photomultiplier tube.

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