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Determination of the dissociation constants (pK_a) of secondary and tertiary amines in organic media by capillary electrophoresis and their role in the electrophoretic mobility order inversion

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Abstract

Non-aqueous capillary electrophoresis (NACE) may provide a selectivity enhancement in separations since the analyte dissociation constants (pK_a) in organic media are different from those in aqueous solutions. In this work, we have studied the inversion in mobility order observed in the separation of tertiary (imipramine (IMI) and amitryptiline (AMI)) and secondary amines (desipramine (DES) and nortryptiline (NOR)) in water, methanol, and acetonitrile. We have determined the pK_a values in those solvents and the variation of dissociation constants with the temperature. From these data, and applying the Van't Hoff equation, we have calculated the thermodynamic parameters ΔH and ΔS. The pK_a values found in methanol for DES, NOR, IMI, and AMI were 10.80, 10.79, 10.38, and 10.33, respectively. On the other hand, in acetonitrile an opposite relation was found since the values were 20.60, 20.67, 20.74, and 20.81 for DES, NOR, IMI, and AMI. This is the reason why a migration order inversion is observed in NACE for these solvents. The thermodynamic parameters were evaluated and presented a tendency that can be correlated with that observed for pK_a values.

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Keywords: Non-aqueous capillary electrophoresis; Selectivity; Dissociation constants; Thermodynamic parameters; Amines

1. Introduction

Non-aqueous capillary electrophoresis (NACE) [1–3] has emerged as an alternative to obtain good separations of hydrophobic analytes without using special additives such as surfactants, cyclodextrins or complexing agents [4–6]. Several studies have been carried out in order to explain the physical chemistry involved in electrophoretic separations where background electrolytes (BGEs) are in presence of organic solvents [7–12]. The first studies have focused on the bulk solvent properties, e.g., viscosity and dielectric constants of organic solvents and how these properties could affect electrophoretic behavior.

In a recent review by Riekkola [2] the properties that affect electrophoretic behavior in NACE were summarized. Briefly, the solvent relative permittivity, ε, is the major factor that governs ion interactions. Solvents with $\varepsilon < 10$ have no practical use since very little or no ionic dissociation takes place. For $10 < \varepsilon < 30$, in which ionic dissociation occurs, ion-pair formation is the dominant effect. The solvent viscosity directly affects the mobility of ions and thus the electroosmosis. However, the viscosity itself cannot be studied separately since changes in solvent composition also affect other medium properties such as the electrical permittivity and the zeta potential [4,13,14].

Other important issue in NACE study is the observation that the analyte ionization constants in organic media are different from those in aqueous solutions. The mobility of the analyte is a function of the mobility of the fully charged species (actual mobility) and its degree of ionization, which, in turn, is a consequence of the dissociation equilibrium. The dissociation equilibrium depends on the thermodynamic stabilization of reagents and products. When shifting from

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aqueous to organic medium the equilibrium is mostly related to the solvent ability in stabilizing charged species. In a series of papers [12,15–18], Kenndler and co-workers reported that the analyte pK_a values are larger in almost all organic solvents commonly used in NACE when compared to aqueous media. The theoretical interpretation for this observation is based on the concept of transfer activity coefficient that consider all species involved in the acid-base equilibrium. A detailed description of such model in the context of CE can be found in [14] and the references cited within.

Since there are several organic solvents with different physical and chemical properties, the pK_a of a single analyte can present different values within quite different scale ranges depending on the medium. Therefore, the selectivity can be enhanced using different pure organic solvents or their mixtures to achieve separations that are impossible to obtain in aqueous media. The pH scale is particular for each solvent and solutions with well-defined pH can be prepared according to the Henderson–Hasselback equation (Eq. (1)) if the pK_a of the BGE in that solvent is known [3].

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{R_3N}]}{[\mathrm{R_3N^+H}]} \tag{1}$$

When studying the electrophoretic separation of aliphatic amines using different aqueous and organic media, an inversion in migration order is observed. An example of this behavior was reported by Bjornsdottir and Hansen [4,7] who separated primary, secondary and tertiary amines with closely related structures. In methanol, dimethylformamide, dimethylacetamide and dimethylsulfoxide media, primary amines eluted first, followed by secondary and tertiary amines. On the other hand, in acetonitrile and *N*-methylformamide the migration order was inversed, i.e., tertiary amines were followed by secondary and primary amines.

A detailed understanding of the dissociation equilibria involved in the inversion phenomena may help to construct a model that allows the prediction of separation behavior in NACE. In this work, we have studied the effect of the dissociation equilibrium on the separation of tertiary (imipramine and amitryptiline) and secondary amines (desipramine and nortryptiline) in water, methanol, and acetonitrile. We have determined the pK_a values in those solvents and the variation of dissociation constants with the temperature. From these data and applying the Van't Hoff equation (Eq. (2)) we have calculated the thermodynamic parameters ΔH and ΔS, which may help to elucidate the inversion in mobility order observed in NACE.

$$\ln K = \frac{\Delta S}{R} - \frac{\Delta H}{R}\frac{1}{T} \tag{2}$$

2. Experimental

2.1. Chemicals

All chemicals and solvents were analytical grade. Methanol (MeOH), acetonitrile (ACN), perchloric acid (70%), and acetic acid were purchased from Merck (Darmstadt, Germany). Ammonium acetate, sodium tetraborate ($Na_2B_4O_7\cdot10H_2O$), and sodium hydroxide were purchased from Mallinckrodt Baker (Xalostoc, Mexico). The amines standards were purchased as hydrochloride salts from different suppliers as follows: imipramine (IMI) and desipramine (DES) from Ciba–Geigy (São Paulo, Brazil), amitryptiline (AMI) from Sigma (St. Louis, USA), and nortryptiline (NOR) from Sandoz (São Paulo, Brazil). Mesityl oxide was obtained from Rhodia (São Paulo, Brazil).

2.2. Sample and BGE solutions

Both analyte stock solutions (2 mg mL^{-1}) and analyte mixtures (100 μg mL^{-1} for AMI and NOR, 50 μg mL^{-1} for IMI and DES) were prepared in methanol and used throughout the work.

For pK_a determination in aqueous medium 20 mM sodium borate buffer solutions were prepared with pH 9.0, 9.5, 10.0, and 10.5. BGEs used were prepared with acetic acid (and ammonium acetate), whose pK_a^* in ACN, and methanol are, respectively, 9.7 and 22.4 [19]. In ACN, the acetic acid concentration was fixed in 1 M and the ammonium acetate concentration was varied from 10 to 70 mM (10, 25, 50 and 70 mM), giving the pH 20.4, 20.8, 21.1, and 22.25, respectively. For methanol the pH of BGE solutions were 10.0 (20 mM acetic acid and 40 mM ammonium acetate), and 10.5 (20 mM acetic acid and 126 mM ammonium acetate).

2.3. Instrumentation and CE procedures

CE experiments were carried out in a HP^{3D}CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector. Analytes were monitored at 214 nm with a bandwidth of 16 nm. A fused silica capillary of 46.5 cm (38 cm to the detection window) × 50 μm i.d. was used and samples were introduced hydrodynamically with 50 mbar by 10 s and separated under 25 kV.

The determination of pK_a by CE is based on the dependence of the analyte electrophoretic mobility as a function of the medium pH. A detailed description of the CE-based method for pK_a determination can be found elsewhere [12,17]. First, the analyte mobilities were measured according to the method by Williams and Vigh [20]. In the first step, a sample plug containing the analyte and the electroosmotic flow (EOF) marker (mesityl oxide) was injected and transferred into the thermostated region of the capillary by applying pressure. It is important to ensure that the electrophoresis will take place within the thermostated region of the capillary since in most of commercial instru-

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