Determination of the retention factor of ionizable compounds in microemulsion electrokinetic chromatography
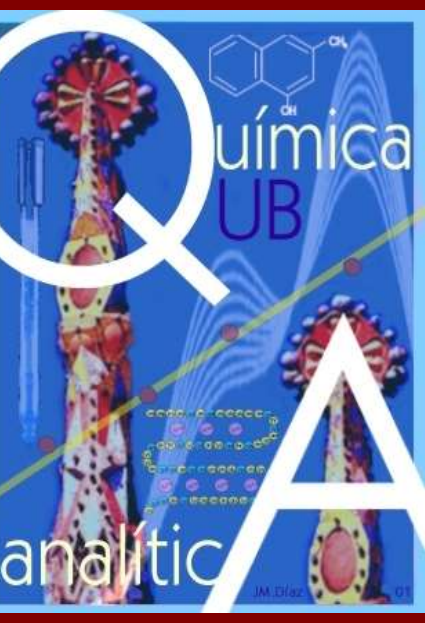
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Introduction

The retention factor (k) of ionized compounds in microemulsion electrokinetic chromatography (MEEKC) is measured through [1]:

$$k = \frac{\mu - \mu_0}{\mu_{ME} - \mu} \quad \text{Eq. 1}$$

- μ: Electrophoretic mobility of the compound in microemulsion.
- μ_0: Electrophoretic mobility of the compound in CZE conditions.
- μ_{ME}: Electrophoretic mobility of the microemulsion marker.

Surfactant (SDS) 1.30% w/v
Cosurfactant (1-butanol) 8.15% v/v
Heptane 1.15% v/v

Figure 1. Schematic representation of the MEEKC pseudostationary phase.

In order to evaluate the partition of ionized compounds into the ME phase, the contribution of the electrophoretic mobility of the compound (i.e. the ionic mobility of the ionized solute caused by the application of an electric field, μ_0) must be subtracted from the observed mobility (μ).

Taking into account that the retention factor of a substance in a given system is often used to estimate other properties of the compounds, such as log $P_{o/w}$ or biopartitioning parameters [2,3], it is very important to ensure its correct determination. The use of two different media can be sometimes an important handicap in the determination of accurate k values. For example, in micellar electrokinetic chromatography (MEKC) inconsistent retention factors, sometimes negative, for ionizable compounds have been found in the literature [4]. This is because in a strict sense, both subtracted media should be the same except for the presence of the ME. However, it is very difficult to simulate the aqueous phase present in a ME or micellar system, so only buffer is used to determine μ_0.

Experimental section

- Instrument: CE Agilent Technologies capillary electrophoresis device equipped with a DAD detector.
- Capillary: fused silica TSP from Polymicro Technologies, 50 µm ID.
- Electroosmotic flow and microemulsion markers: DMSO and dodecanophenone, respectively.

- Injection: 5s 50 mbar.
- Separation conditions: 25 ºC, 8-15 kV.
- Buffer in CZE: Phosphate or acetate buffer at 50 mM ionic strength.
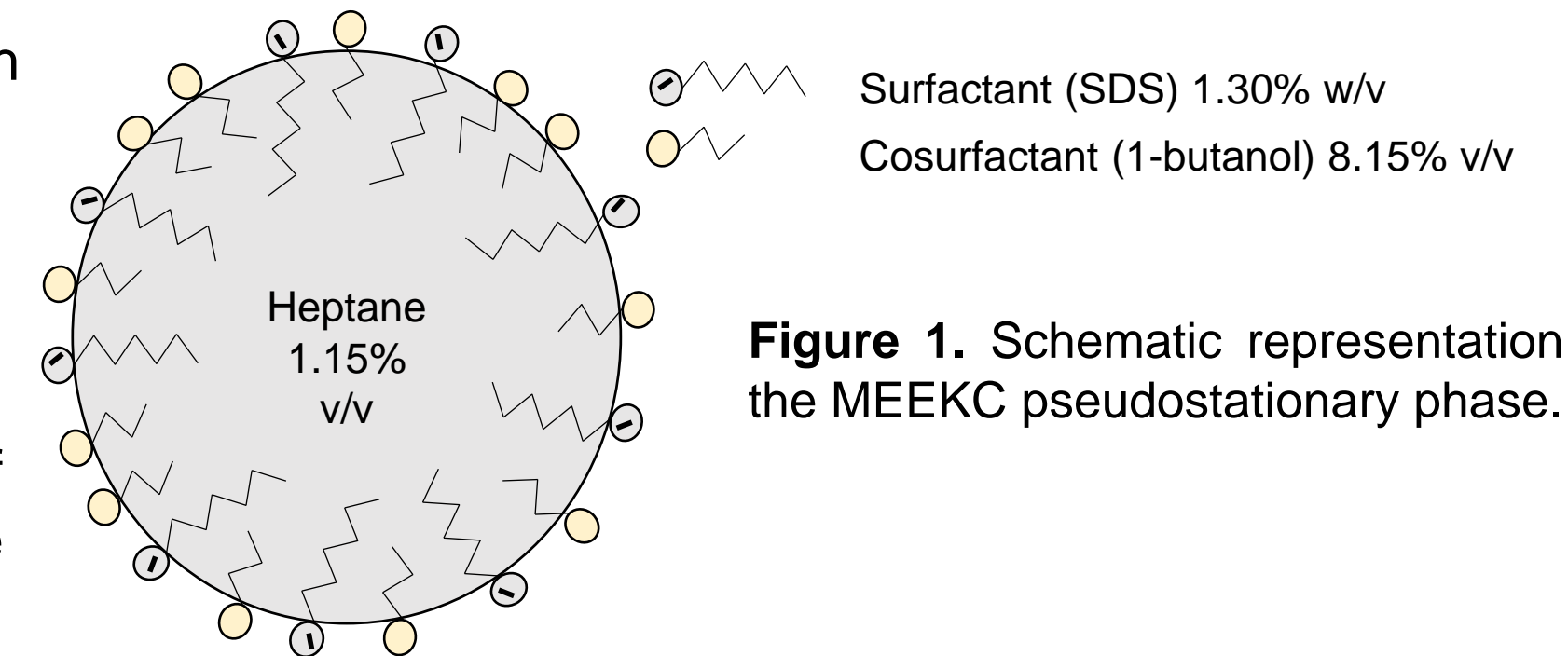- Microemulsion [2] (see Figure 1)

Results and discussion

The retention factor of six ionizable acids, with a pK_a between 3.5-6.5, has been calculated using Eq. 1 at several pH values ranging from 2.0 to 8.0. μ_0 has been measured in plain buffer. Then, k-pH profiles have been calculated, adjusting the data to the following equation:

$$k = \frac{k_{(HA)} + k_{(A-)} \cdot 10^{pH - pK'_a}}{1 + 10^{pH - pK'_a}} \quad \text{Eq. 2}$$

- $k_{(HA)}$: Retention factor of the protonated acid.
- $k_{(A-)}$: retention factor of the fully ionized acid.
- pK_a': Apparent acidity constant.

The k-pH profiles obtained from the fit of Eq. 2 to experimental data are shown in Figure 2 (red lines). Often, negative k values have been obtained, especially when the acids are highly or fully ionized.
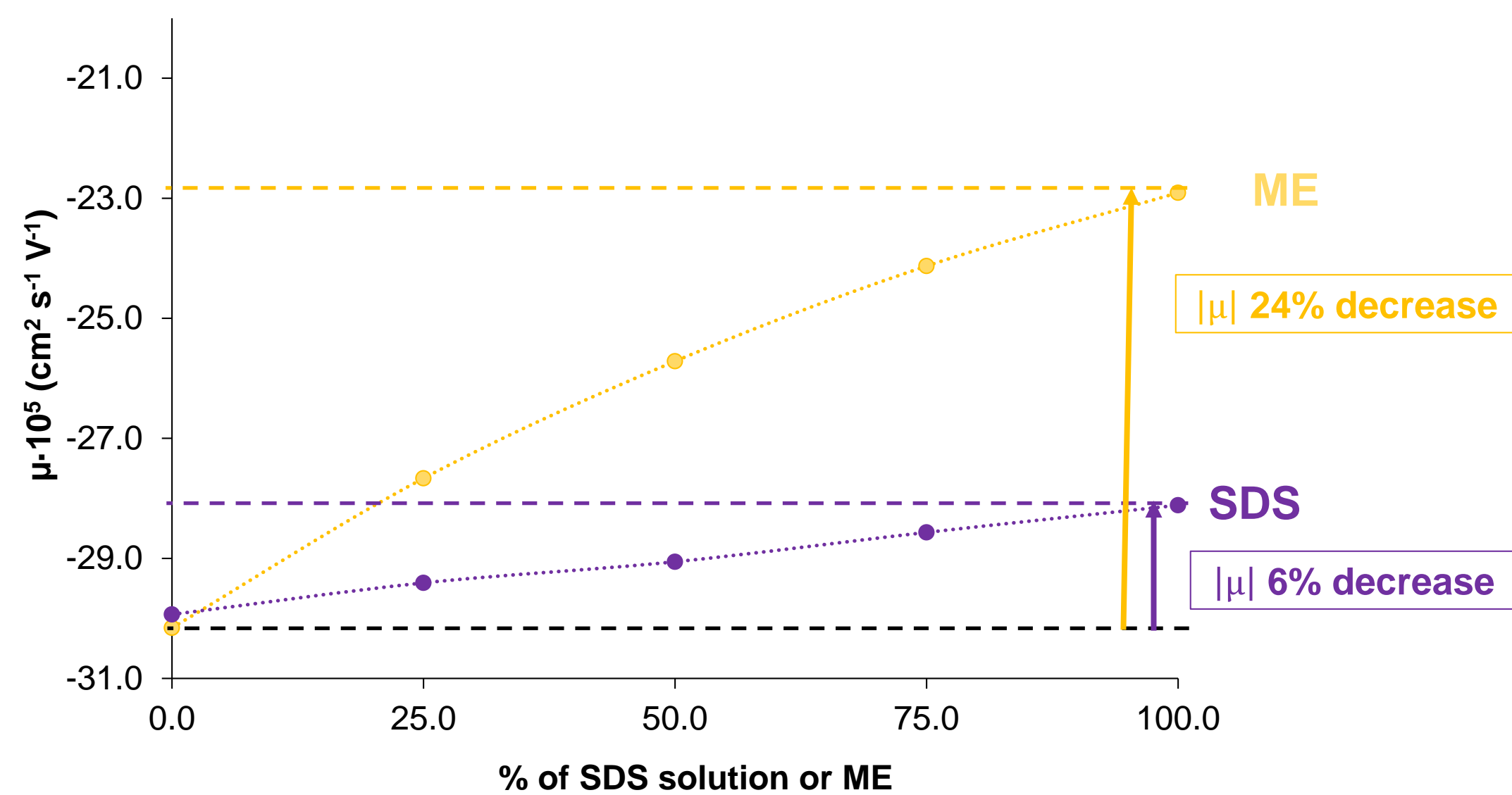

Figure 3. Effect of the amount of ME (yellow) or SDS (purple) on the mobility of benzoate ion.

In Figure 3, µ of benzoate ion, a ME non-interacting compound, has been measured at different proportions of an SDS solution 1.30% w/v and of the ME. µ varies depending on the medium and the components concentration. Since µ and η are inversely related, differences in viscosities between the solutions used may lead to inaccurate k measurements especially when ME are used, since 1-butanol increases more the viscosity of the medium than SDS (Figure 4).
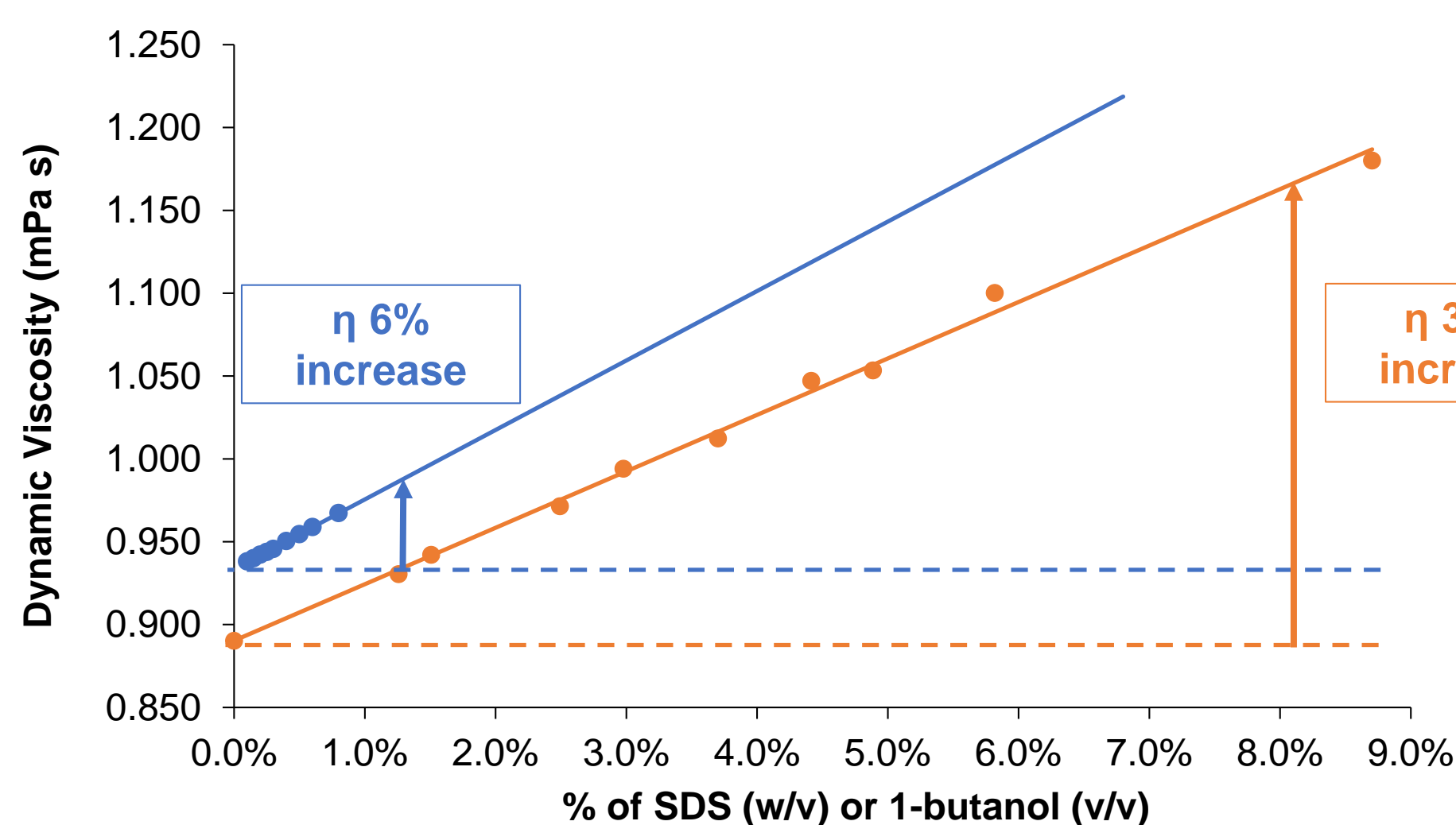

Figure 4. Effect on viscosity of SDS (blue; [NaCl]=0.05M) and 1-Butanol (orange).

To solve this problem, a viscosity correction factor is introduced in Eq. 1, leading to Eq. 3.

$$k = \frac{\mu - \left(\frac{\mu}{\mu_0}\right)_{benzoate} \cdot \mu_0}{\mu_{ME} - \mu} \quad \text{Eq. 3}$$

- (μ/μ_0) consists on the ratio of the mobilities of a ME non-interacting compound (such as benzoate ion).

Therefore, k-pH profiles have been recalculated through Eq. 3 (Green lines in Figure 2), leading to accurate results

Figure 2. k vs. pH profiles of the six test compounds in MEEKC, before (red line) and after (green line) viscosity correction.

References

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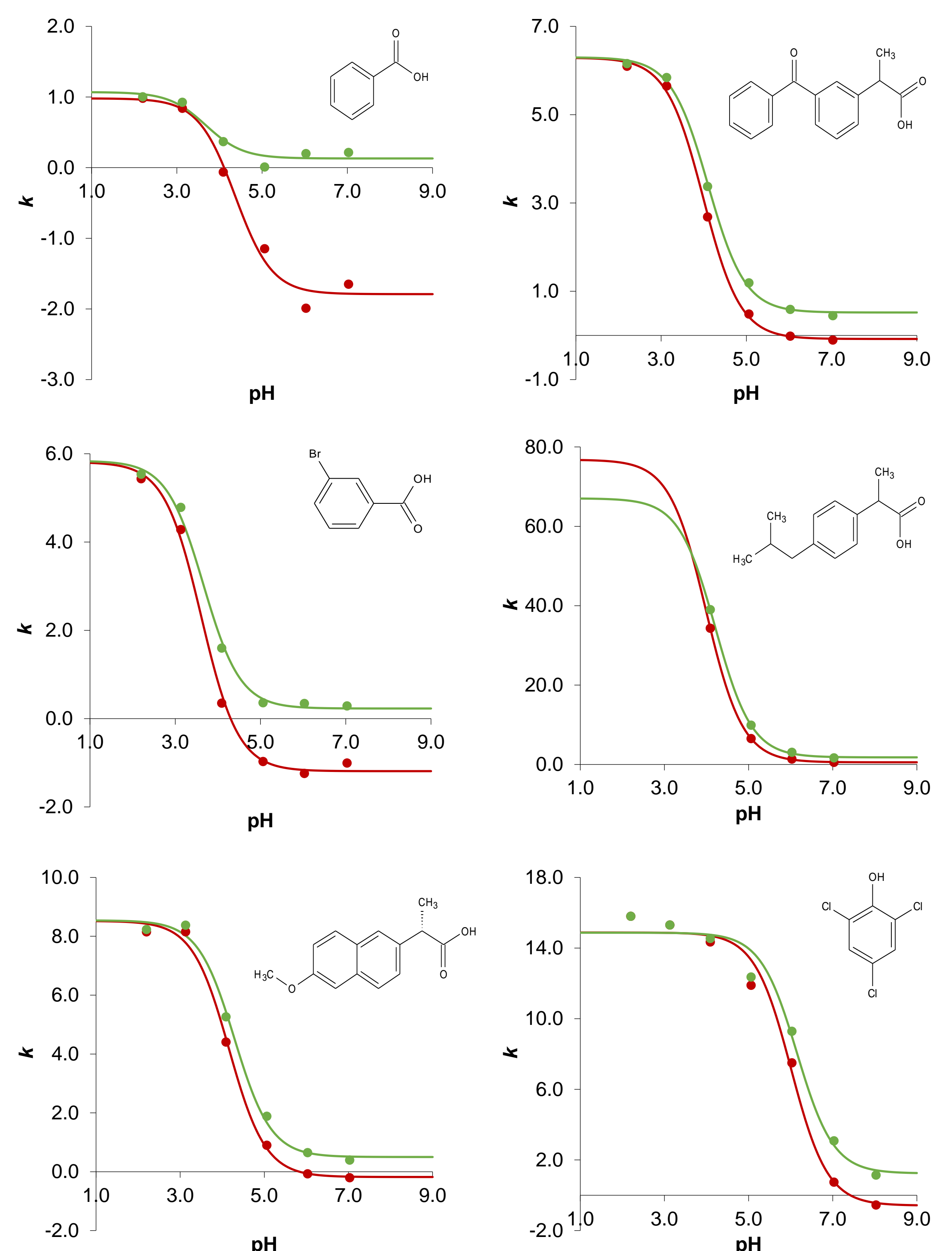[4] C.E. Lin, W.C. Lin, W.C. Chiou. J. Chromatogr. A., 1996, 722, 333–343.

Conclusions

- Viscosity affects µ of ionizable compounds in MEEKC in a big extent.
- CZE buffer-medium has a lower viscosity than microemulsion buffer-medium (by 24%)
- Viscosity difference can be corrected by mobility measurements of an unretained compound.