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Review

Effect of temperature on acid–base equilibria in separation techniques. A review

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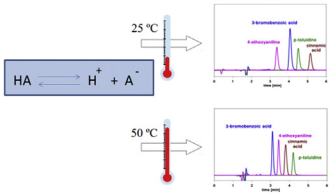
HIGHLIGHTS

- The study of theoretical principles of acid–base equilibrium has been reviewed.
- The proton transfer process is often present in the analytical separation practice.
- The influence of temperature on secondary chemical equilibria is examined.
- The focus is laid on liquid chromatography and capillary electrophoresis.
- Temperature can be a useful variable to modify selectivity under predictable basis.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies on the theoretical principles of acid–base equilibria are reviewed and the influence of temperature on secondary chemical equilibria within the context of separation techniques, in water and also in aqueous-organic solvent mixtures, is discussed. In order to define the relationships between the retention in liquid chromatography or the migration velocity in capillary electrophoresis and temperature, the main properties of acid–base equilibria have to be taken into account for both, the analytes and the conjugate pairs chosen to control the solution pH. The focus of this review is based on liquid–liquid extraction (LLE), liquid chromatography (LC) and capillary electrophoresis (CE), with emphasis on the use of temperature as a useful variable to modify selectivity on a predictable basis. Simplified models were evaluated to achieve practical optimizations involving pH and temperature (in LLE and CE) as well as solvent composition in reversed-phase LC.

Contents

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1. Introduction

The concept of an acid or a base underwent considerable changes during the twentieth century. Thus, the term "acid" encompass not only "hydrogen acids" but also numerous nonhydrogen-containing species, whereas the term "base" has become greatly broadened by the inclusion of all molecules able to interact with a hydrogen ion (*e.g.*, those having electron-donor atoms like N or O, and/or π-electrons). The huge number of articles currently found under the combination of the search terms "acid" with other key words, such as "temperature" or "separation techniques", testifies to the necessity of formulating this term correctly. The interactions specifically treated here will refer strictly to acids and bases according to the Brønsted–Lowry concept, *i.e.*, the transfer of a proton from a donor (acid) to an acceptor (base) compound with the consequent change in ionic charges of both species.

An understanding of acid–base equilibria is an essential part of the chemical knowledge. Since the physicochemical properties of neutral and ionized forms are different, the pK_a of weak acids and bases is a key value. Thus, the extent of ionization will determine properties of these compounds, such as absorption, distribution, or transport in biological and environmental systems [1]. Similarly, acid–base equilibria plays a major role in several analytical techniques and, especially, in separation science [2]. Therefore, since the temperature is fundamental in proton transfer reactions, this parameter will undoubtedly affect the ionization equilibria involved in many separation techniques.

The aim of this review is to provide a concise discussion based on representative results taken, partly from work reported in the literature, and partly from experimental work conducted by the authors and associates. The contents of this review article are outlined as general thermodynamic fundamentals on acid–base equilibria, and the effect of temperature on these equilibria with a particular emphasis on separation techniques, mainly liquid–liquid extraction (LLE), liquid chromatography (LC) and capillary electrophoresis (CE).

In recent years, the scientific community has taken an increased interest in the use of hydrophilic interaction liquid chromatography (HILIC) for the separation of a wide variety of samples containing small polar compounds [3,4] and usually temperature is considered a useful variable for fine tuning resolution. The complexity of the retention mechanism in HILIC makes the interpretation of many results difficult. In addition, the acid–base equilibria and all the variables affecting the dissociation constants of buffer components and solutes can be relevant. The interpretation can be even more complex if one takes into account that (i) most solutes are weak electrolytes and, (ii) mobile phases contains a buffer in a highly organic fraction.

Finally, several relevant practical considerations about the deliberate implementation of temperature in liquid chromatography and in electrophoresis are critically discussed. The times in which chromatographers were afraid of the column integrity upon heating are gone. Nowadays, the availability of more chemically and thermally stable chromatographic supports has overcome this drawback and changed our perspective. Otherwise, from the point of view of the complexity of real samples, the consideration of temperature as another leading variable provide an additional degree of freedom for enhancing selectivity and achieving success in resolution.

2. Theory

2.1. Thermodynamic considerations

The acid–base equilibrium of a monoprotic acid HA^z in a given solvent S can be expressed as:

$$HA^z + nS \rightleftarrows S_nH^+ + A^{z-1} \quad (1a)$$

where n denotes the minimum number of solvent molecules able to carry the proton, and S_nH^+ refers to the association of solvated hydrogen ion. This is usually simplified to:

$$HA^z \rightleftarrows H^+ + A^{z-1} \quad (1b)$$

where z corresponds to the specie's charge before dissociation, H^+ denotes the (solvated) hydrogen ion. The thermodynamic acid dissociation constant, K_a, for both equilibria can be defined by the equation

$$K_a = \frac{a_A a_H}{a_{HA}} \quad (2)$$

where a_i refers to the activity of the species i: hydrogen ion, deprotonated A^{z-1} and the acidic form HA^z. Throughout this review the designation of HA and A will refer to the acidic and basic forms of the conjugate pair, respectively, and the charges will be omitted in the superscripts for the sake of simplicity. The value of K_a under specified conditions of solvent, temperature and pressure depends on the concentration scale. In analytical chemistry, molarity is widely used because solutions of accurately known concentration can be conveniently prepared with volumetric glassware and standardized by volumetric methods. Nevertheless, for some purposes—*i.e.*, experiments dealing with temperature changes or mixtures of components where volumes are not additive—concentration scales given in moles and weights, are more convenient. Most of the equilibrium constants, electrode potentials and thermodynamic data compiled from the literature correspond to values in the molal scale. Since at 25 °C and 1 atm of pressure the density of water is 0.99707 g/ml, molar and molal concentration scales are nearly the same if water is the solvent. For diluted solutions the difference between both scales is about 0.001 pK_a units, a difference that in most analytical situations can be ignored. Unless specifically indicated, the molal scale will be used throughout this review.

The activities are related to concentrations by $a_i = m_i\,\gamma_i$ where m_i and γ_i represent the molality and the activity coefficient of the species i, respectively. The magnitude of γ_i, for electrolytes and non-electrolytes, is a measurement of the deviation from ideal behavior [5].

2.1.1. Influence of temperature on activities and dissociation constants

In accordance with conventions, the standard state is chosen at the temperature of interest. In the molal scale, the effect of temperature on the activity will be a consequence of the effect of temperature on the activity coefficient. For ionic species in dilute solutions, the change of a_i caused by temperature can be calculated from the change of coefficients in the Debye-Hückel equation, whose expression for molal concentration scales can be given as follows:

$$-\log \gamma_i = \frac{z_i^2 A\sqrt{I}}{1 + a_0 B\sqrt{I}} \tag{3}$$

where z is the charge of the i ion, and I denotes the ionic strength ($I = \sum_j^n m_j z_j^2$ where m_j and z_j refers to molal concentration and charge of the ions in solution) and, A and a_0B are two solvent- and temperature-dependent parameters that can be estimated by following the Bates–Guggenheim convention [6–9].

Moreover, the dependence of any equilibrium constant on temperature is given by the van't Hoff equation. For the acid–base equilibria ruled by K_a, the equation is written as,

$$\frac{\mathrm{d}\,\ln K_a}{\mathrm{d}T} = \frac{\Delta H_a^0}{RT^2} \tag{4}$$

In this equation, ΔH_a^0 denotes the change in standard enthalpy for the dissociation of HA, and R is the universal gas constant. Its integration within a finite temperature range requires a knowledge of the dependence of ΔH_a^0 on temperature. Clarke and Glew [10] proposed an approach to the treatment of K_a–T data considering $K_a(T)$ as a continuous function of temperature and assuming that the related thermodynamic function changes are also well behaved functions of T. Once a reference temperature (θ) is chosen, ΔH_T^0, $\Delta C_{p,T}^0$, and heir successive derivatives can be expressed as a perturbation of their values at the reference temperature θ by using a Taylor-series expansion for each term. The equation is given by

$$\ln K_a(T) - \ln K_a(\theta) = \frac{-\Delta H_\theta^0}{R}\left[\frac{1}{T} - \frac{1}{\theta}\right] + \frac{\Delta C_{p,\theta}^0}{R}\left[\frac{\theta}{T} - 1 + \ln\frac{T}{\theta}\right] + \frac{1}{R}\frac{\partial \Delta C_{p,\theta}^0}{\partial T}\ldots \tag{5}$$

This function permits the estimation of the acid–base thermodynamic parameters along with their standard deviations from

Table 1
pK_a and dissociation thermodynamic functions of usual substances used to prepare buffer solutions in water.

Compound	pK_a	ΔG_a^0 (kJ mol^{-1})	ΔH_a^0 (kJ mol^{-1})	ΔC_{pa}^0 (JK^{-1} mol^{-1})
Tartaric acid (pK_{a1})[a]	3.036	17.33	3.19	−147
Citric acid (pK_{a1})[a]	3.128	17.855	4.07	−131
Phthalic acid (pK_{a2})[a]	5.408	30.869	−2.17	−295
Phosphoric acid (pK_{a2})[a]	7.198	41.087	3.6	−230
Borax[a]	9.237	52.725	13.8	−240
Carbonic acid (pK_{a2})[a]	10.329	58.958	14.7	−249
ACES[b]	6.847	39.083	30.43	−49
CABS[b]	10.499	59.929	48.1	57
CAPSO[b]	9.825	56.082	46.67	21
CHES[b]	9.394	53.621	39.55	9
HEPES[b]	7.564	43.176	20.4	47
HEPPS[b]	7.957	45.419	21.3	48
HEPPSO[b]	8.042	45.904	23.7	47
MES[b]	6.27	35.789	14.8	5
MOPSO[b]	6.90	39.39	25	38
Formic acid[c]	3.10	17.69	−0.37	–
Acetic acid[c]	4.756	27.15	−0.41	−142
Ammonia[c]	9.245	52.77	51.95	8
1-Methylpiperidine[c]	10.08	57.53	39.50	–
Pyrrolidine[c]	11.30	64.50	53.64	–
Tris-(hydroxymethyl)-aminomethane[c]	8.072	46.075	47.45	−59
Triethanolamine[c]	7.762	44.306	33.6	50
Triethylamine[c]	10.72	61.19	43.13	151

Data taken from Refs. [22,23].
[a] NBS buffers [16].
[b] Good's buffers [17–19].
[c] buffers recommended for MS detection.

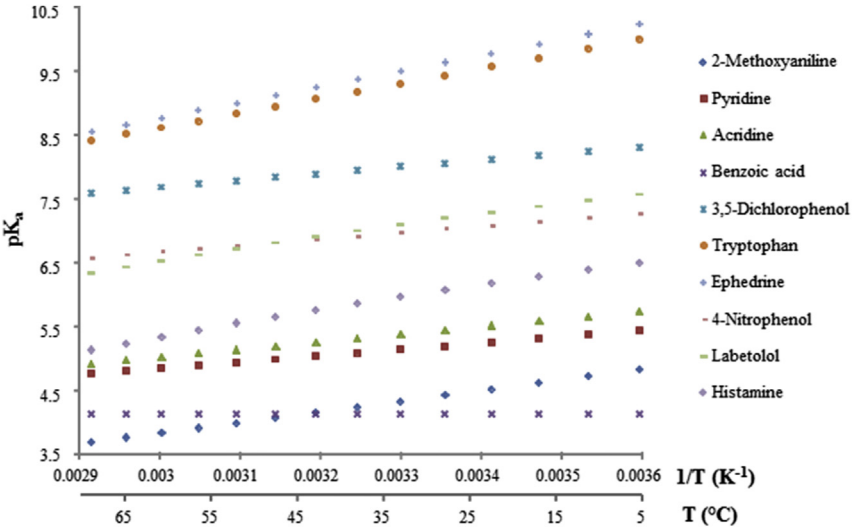

Fig. 1. Plots of ${}^{w}_{w}pK_a$ as a function of temperature corresponding to several representative compounds. Data taken from Refs. [22] and [24].

least-square regressions. Originally applied to describe the dissociation of aqueous hydrogen cyanide within a temperature range, the approach has been applied to the interpretation of other acid–base systems [11–15].

The third and successive terms of the series increase in relative significance as the temperature difference with respect to θ increases. A widely used simplification consists in assuming that ΔH_a^0 is independent of temperature over the temperature range of interest, then Eq. (4) becomes:

$$\ln K_a(T_2) - \ln K_a(T_1) = \frac{-\Delta H_a^0}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right] \quad (6)$$

where 2 and 1 refer to two (target and reference) temperatures. This equation predicts that the logarithm of K_a changes linearly with the reciprocal temperature, which is an acceptable assumption within a narrow temperature range. Nevertheless, for an extended range this approximation can result somewhat rough. For example, the K_a of most carboxylic acids in water first increases and then decreases as temperature goes from 0 to 100 °C. Alternatively, if ΔC_p^0 is available and is independent of temperature, Eq. (4) can be integrated by considering only two terms of the Taylor series. Then, the integration between those two temperatures results in:

$$\ln K_a(T_2) - \ln K_a(T_1) = \frac{\Delta H_a^0}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right] - \frac{\Delta C_p^0}{R}\left[\frac{T_2}{T_1} - 1 + \ln\frac{T_1}{T_2}\right] \quad (7)$$

Table 1 summarizes the values of the pK_a, dissociation enthalpies and heat capacities of representative acids, bases and amphoteric compounds. Thermodynamic dissociation properties of substances recommended for preparing calibration buffers [16], Good's buffers [17–19] and those used to prepare buffers in LCMS [20,21] are compiled. Fig. 1 provides a plot of the dependence of pK_a on reciprocal temperature for representative solutes from different families of compounds [22–24].

2.1.2. Solvent dependence

For a long time, the chemistry of electrolytes was studied almost exclusively in aqueous solutions and, thus, conventions where solute activities were referred to their aqueous standard states were naturally adopted. Later on, in analytical chemistry, nonaqueous miscible solvents have provided an additional means of shifting chemical equilibria so as to effect analyte separation or identification. Both the properties of solutes and the chemical equilibria in the solution will in some way be affected by changing the solvent, so that sometimes confusion can result from the arbitrariness of the standard states chosen. For analytical purposes, the choice of a different reference state for each solvent is generally preferable—namely, the infinitely dilute solution of the solute in that solvent. This choice allows the activity of the solute in any solvent to be numerically equal to concentration if the solution is sufficiently dilute. The convention implies that $a = 1$ in any solvent and $G = G^0$ in any solvent. Thus, the activity of a solute i can be referred to its standard state either in the nonaqueous solvent

$$G_i = {}^{s}_{s}G_i^0 + RT \ \ln {}^{s}_{s}a_i \quad (8)$$

or in water,

$$G_i = {}^{s}_{w}G_i^0 + RT \ \ln {}^{s}_{w}a_i \quad (9)$$

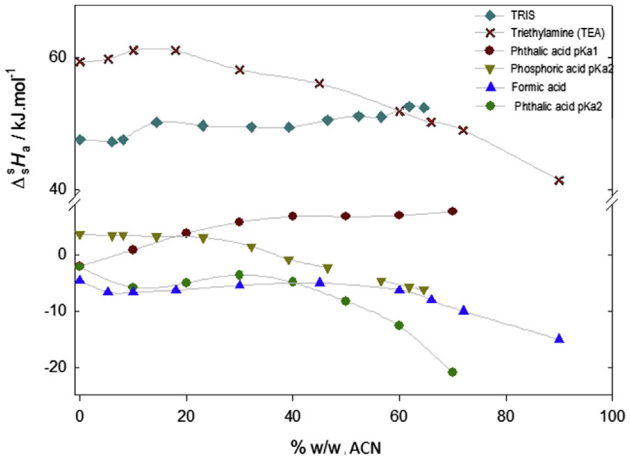

Fig. 2. Plots of $\Delta^s_s H_a^0$ as a function of % (w/w) acetonitrile for compounds used for buffer preparation. Data taken from Refs. [13,27,28].

where G_i denotes the partial molar Gibbs free energy of solute i in the given solvent and ${}^{s}_{s}G^{0}_{i}$ and ${}^{s}_{w}G^{0}_{i}$ are the standard partial molar Gibbs free energies of the solute in the nonaqueous solvent and in water, respectively. According to the IUPAC [9,25], the left-hand superscript indicates the measurement solvent, whereas the left-hand subscript denotes the chosen standard state solvent. The activities are related to molality, m_i, through the corresponding activity coefficient, γ_i, by ${}^{s}_{s}a_i = m_i {}_{s}\gamma_i$ and ${}^{s}_{w}a_i = m_i {}_{w}\gamma_i$, in which equation ${}_{s}\gamma_i$ and ${}_{w}\gamma_i$ become unity at infinite dilution in the organic mixture and in water, respectively. By combining Eqs. (8) and (9), and substituting a_i with the product of m_i and γ_i, the following equation results:

$$ {}^{s}_{s}G^{0}_{i} - {}^{s}_{w}G^{0}_{i} = \Delta_t G^{0}_{i} = RT \ln \gamma_{t,i} \tag{10} $$

where $\Delta_t G^{\circ}$ stands for the change in Gibbs free energy upon transfer of 1 mol of the species i from the standard state in water to the standard state in the organic solvent and $\gamma_{t,i}(={}_{w}\gamma_i/{}_{s}\gamma_i)$ represents the primary medium effect. Operationally, $\gamma_{t,i}$ can be considered as a conversion factor from one scale to the other:

$$ {}^{s}_{w}a_i = {}^{s}_{s}a_i \gamma_{t,i} \tag{11} $$

2.2. Acid–base equilibria in aqueous-organic solvents

An acid–base dissociation can be considered as a sequence of three thermodynamic steps: (i) the transfer of the acid from the solvated to the gas phase; (ii) dissociation of the acid into ions and (iii) the solvation of these ions in the given solvent. The nature of the solvents will affect the first and the last steps, mainly as a consequence of specific solute–solvent interactions (solvation effects) and of electrostatic interactions. Both transitions are determinants of the pK_a value. Thus, in solvents different from water, the proton-transfer equilibrium as defined in Eq. (1) assumes negligible ion pairing, which is reasonable for dilute compounds and solvent mixtures of relatively high dielectric constant. Extensive studies were conducted by Bosch et al., who thoroughly examined the effect of different solvents on aqueous pK_a values for a large number of acids and bases, and mainly those employed to prepare buffer solutions. A representative review of this work can be found in Ref. [26].

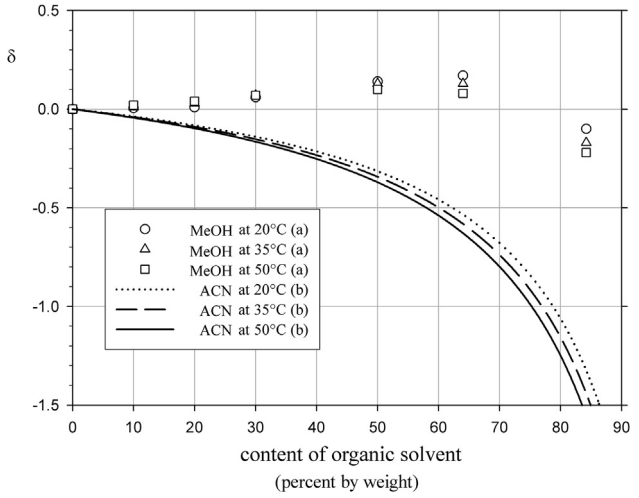

Fig. 3. Conversion parameter between pH scales (${}^{s}_{w}$pH and ${}^{s}_{s}$pH) at different compositions by weight, for MeOH/water and ACN/water mixtures and at three temperatures. Adapted from Refs. [33,34].

The standard free energy of the dissociation of HA in the system can be expressed with reference to the state of the solvent mixture

$$ \Delta^{s}_{s}G^{0}_{a} = -RT \ln {}^{s}_{s}K_a = \Delta^{s}_{s}H^{0}_{a} - T\Delta^{s}_{s}S^{0}_{a} \tag{12a} $$

or to the aqueous standard state

$$ \Delta^{s}_{w}G^{0}_{a} = -RT \ln {}^{s}_{w}K_a = \Delta^{s}_{w}H^{0}_{a} - T\Delta^{s}_{w}S^{0}_{a} \tag{12b} $$

where ΔG^{0}_{a}, ΔH^{0}_{a} and ΔS^{0}_{a} are the standard Gibbs free energy, enthalpy and entropy for the proton-transfer equilibrium of HA in the solvent mixture s—referred either to the solvent mixture (s) or to water (w) as the standard state solvent for determining the activity coefficients—and K_a is the acid–base dissociation constant referred to the solvent mixture,

$$ {}^{s}_{s}K_a = \frac{{}^{s}_{s}a_{H}\,{}^{s}_{s}a_{A}}{{}^{s}_{s}a_{HA}} \tag{13} $$

or to water,

$$ {}^{s}_{w}K_a = \frac{{}^{s}_{w}a_{H}\,{}^{s}_{w}a_{A}}{{}^{s}_{w}a_{HA}} \tag{14} $$

a_i is the activity of a species i on the corresponding solvent reference scale.

At constant pressure, the effect of temperature can be obtained from the van't Hoff equation as:

$$ \frac{\mathrm{d}\ln {}^{s}_{s}K_a}{\mathrm{d}(1/T)} = -\frac{\Delta^{s}_{s}H^{0}_{a}}{R} = -\frac{1}{R}\left[\Delta^{s}_{w}H^{0}_{a} - \left(\Delta_t H^{0}_{HA} - \Delta_t H^{0}_{H} - \Delta_t H^{0}_{A}\right)\right] \tag{15} $$

where $\Delta_t H^{0}_{i}$ represents the enthalpy of transfer of 1 mol of compound i in its standard state from water to the solvent mixture. Eq. (15) assumes that enthalpies are temperature-independent. Fig. 2 shows the dependence of $\Delta^{s}_{s}H^{0}_{a}$ data for the ionization of four acids and two bases, usually employed for buffer preparation, on acetonitrile/water composition [27,28].

2.2.1. Measurement of pH in aqueous-organic mixtures

The thermodynamic quantities, ${}^{s}_{w}$pH and ${}^{s}_{s}$pH—two different ways to define the activity of H^+ ions in solvent mixtures depending on the chosen standard state— are related as follows

$$ {}^{s}_{s}\mathrm{pH} = -\log {}^{s}_{s}a_{H} = {}^{s}_{w}\mathrm{pH} - \log\ \gamma_{t,H} \tag{16} $$

Two methodologies can be used to determine the pH of a buffer solution in aqueous-organic mixtures: (i) measurement of pH in the given solvent with a pH electrode system calibrated with aqueous buffers (${}^{s}_{w}$pH) and; (ii) measurement of pH after calibrating the electrode system with reference buffers prepared in the same solvent mixture (${}^{s}_{s}$pH). When the pH is measured with a glass combination electrode, a liquid-junction potential is established between the internal salt bridge and the external solution. When the calibration and measurement are both carried out in aqueous solutions, the liquid-junction potentials are similar and therefore cancel out. When the calibration is performed in an aqueous solution and the pH is measured in other solvents these potentials are different. The residual liquid-junction potential, $\overline{E}_j$ accounts for that difference, and the value remains on the intersolvent pH scale. Thus, the difference between the two scales, δ, is [8,29,30].

$$ \delta = {}^{s}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH} = \overline{E}_j - \log \gamma_{t,H} \tag{17} $$

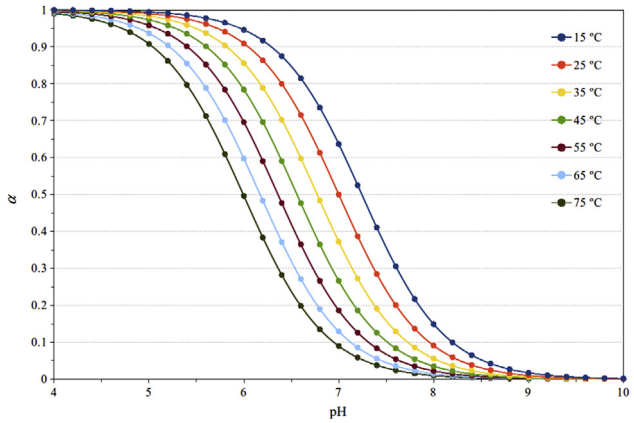

Fig. 4. Degree of dissociation as a function of pH at different temperatures. Hypothetical compound with $pK_a(25\ ^\circ C) = 7$ and $\Delta H^\circ_a = 40$ kJ mol^{-1}

Bates and De Ligny and coworkers have demonstrated that the difference (δ) between these two terms is a constant characteristic of each solvent mixture and temperature, and independent of the buffer components at a given solvent composition. Values for the δ parameter have been measured for several mixed solvents at 25 °C with either hydrogen electrodes [7,8,31,32] or combined glass electrodes as indicator electrodes [26]. Similarly, values of the δ parameter in methanol/water mixtures at temperatures in the range from 20 to 50 °C [33] and in acetonitrile/water mixtures between 20 and 60 °C [34] have likewise been determined. Fig. 3 depicts the results of all these values. The practical significance of the δ parameter can be appreciated from the following consideration: ${}^{s}_{s}pH$ is obtained by measuring ${}^{s}_{w}pH$ with aqueous standards and subtracting the appropriate value of δ for the particular solvent composition and temperature.

2.3. Temperature in liquid-phase separations

The introduction of a reversible equilibrium into a separation method (the so-called secondary chemical equilibrium) provides a very useful option for controlling the separation of individual components [2,35–37]. Secondary chemical equilibria have been widely used to facilitate separations in many systems based on either phase-distribution or migration processes. Acid–base equilibria provide some of the simplest examples of the use of these secondary chemical equilibria [2]. In liquid–liquid extraction, the pH can be used to separate organic acids from salts, or to promote masking reactions followed by liquid–liquid separations. Moreover, the pH of the medium controls the net charge of a given ionic compound, which in turn controls that compound's direction and velocity of migration in electrophoretic methods. In addition, the pH has been used to control retention in many liquid chromatographic techniques. In all these methodologies, the solvent pH determines the degree of dissociation of the weak electrolyte. The fraction, α, is defined as the ratio between the equilibrium concentration of the species HA and the total concentration:

$$\alpha = \frac{[HA]}{m_{HA}} = \frac{1}{1 + (K_a/a_H)(\gamma_{HA}/\gamma_A)} \cong \frac{1}{1 + 10^{(pH - pK_a)}} \tag{18}$$

Now, if the pH of the solution is controlled by the pair HB^z/NaB^{z-1}, whose equilibrium is,

$$HB^z \rightleftarrows B^{z-1} + H^+ \quad K_{a,B} = \frac{a_H a_B}{a_{HB}} \tag{19}$$

$$\Delta G^0_{a,B} = -RT \ln\ K_{a,B} = \Delta H^0_{a,B} - T\Delta S^0_{a,B} \tag{20}$$

where $K_{a,\,B}$ is the acidity constant of the buffer compound (with the ion charges omitted). The pH can be obtained from:

$$pH = pK_{a,B} - \log\frac{a_{HB}}{a_B} = pK_{a,B} + \log\frac{[B^{z-1}]}{[HB^z]} + \log\frac{\gamma_B}{\gamma_{HB}} \approx pK_{a,B} + \log\frac{m_B}{m_{HB}} \tag{21}$$

where [HB] and [B] denote the equilibrium concentrations and m_{HB} and m_B are the total molal concentrations of the buffer components. The last approximation on the right-side of Eq. (21), known as Henderson-Hasselbach equation, is valid under two assumptions: (i) the term $\log\frac{\gamma_B}{\gamma_{HB}}$ was considered close to zero and, (ii) the buffer is sufficiently concentrated and the pH of the mobile phase is far from 0 and from the pK_{ap} (K_{ap} being the autoprotolysis constant of the solvent), thus equilibrium concentrations are close to total concentrations. Upon substitution of Eq. (21) in Eq. (18), the following equation results:

$$\alpha = \frac{1}{1 + (m_B/m_{HB})10^{(pK_{a,B} - pK_a)}} \tag{22}$$

From the thermodynamics of the ionization of solute A and buffer B, expressed respectively in Eq. (4) and Eq. (20), the following expression can be obtained:

$$\alpha = \frac{1}{1 + (m_B/m_{HB}) \times \exp\left[\frac{(\Delta H^0_a - \Delta H^0_{a,B})}{RT}\right] \exp\left[\frac{\Delta(\Delta S^0_a - \Delta S^0_{a,B})}{R}\right]} \tag{23}$$

Eq. (23) clearly shows that the analyte's fractions HA and A at different temperatures will change according to the ionization enthalpies of both HA and buffer B. This expression assumes that m_B/m_{HB} is almost constant. In Fig. 4, the fraction α is plotted as a function of pH for a hypothetical compound of $pK_a = 7$ and $\Delta H^0_a = 40$ kJ/mol at different temperatures.

3. Liquid–liquid extraction

The distribution of a weak acid, HA, in an immiscible organic solvent and an aqueous system, can be written as follows:

$$HA_w \rightleftarrows HA_{org} \quad K = \frac{a_{HA(org)}}{a_{HA(w)}} \tag{24}$$

where w and org refer to the aqueous and organic phases, respectively, and K denotes the distribution constant; expressed as the ratio of activities of HA in both phases in the equilibrium. The standard free energy for the distribution processΔG^0, is expressed as

$$\Delta G^0 = -RT\ln K = \Delta H^0 - T\Delta S^0 \tag{25}$$

where ΔH^0 and ΔS^0 denote the corresponding standard enthalpy and entropy for the distribution of HA between the phases. The distribution ratio, D, is defined as the ratio of the total concentrations of HA, C_{HA}, in both phases:

$$D = \frac{C_{HA(org)}}{C_{HA(w)}} \tag{26}$$

In the organic phase and assuming that: (i) solutions are diluted and, (ii) HA does not associate to form dimers, $C_{HA(org)} \approx a_{HA(org)}$. In

the aqueous phase, the acid–base equilibrium will be dictated by the dissociation constant. Therefore, substitution of Eq. (18) into Eq. (26) leads to:

$$D = \frac{K}{1 + K_a/a_H} = \alpha K \tag{27}$$

expression that enables an evaluation of the distribution ratio D within the whole pH range of the aqueous phase on the basis of the corresponding values for the distribution constant and α.

From the thermodynamics of the ionization of solute A and buffer B, expressed in Eq. (23), and the thermodynamics of distribution of HA between both immiscible phases [*i.e.*, Eq. (19)], the following expression can be obtained:

$$D = \frac{\exp\left[\frac{-\Delta H^0}{RT}\right] \times \exp\left[\frac{\Delta S^0}{R}\right]}{1 + (m_B/m_{HB}) \times \exp\left[\frac{-\left(\Delta H^0_a - \Delta H^0_{a,B}\right)}{RT}\right] \times \exp\left[\frac{\left(\Delta S^0_a - \Delta S^0_{a,B}\right)}{R}\right]} \tag{28}$$

Eq. (28) clearly shows that the distribution of an analyte at different temperatures will change, not only according to the distribution coefficient, ruled by the sign of ΔH^0, but also on the basis of the ionization enthalpies of both HA and buffer B.

Although liquid–liquid extraction (LLE) is an old technique, the effect of temperature on liquid–liquid extraction has been scarcely considered. Robak et al. [38] have recently discussed the distribution of 8-hydroxyquinoline and its derivatives, all of which compounds are diprotic, between water and immiscible solvents within temperatures ranging from 20 to 50 °C and applied linear solvation energy relationship analysis to the data. The distribution constants of those compounds were measured in thirty solvents and at several temperatures and the data were modeled in terms of properties of the solute, solvatochromic properties of the organic solvent, ionic strength of the aqueous phase, and temperature. The model, however, possessed a moderate predictive ability respect to temperature changes. This is expected since the dissociation constants K_{a1} and K_{a2} of such diprotic compounds are also temperature-dependent.

Organic acids can be extracted from different aqueous solutions (*e.g.*, streams of industrial waste, or bio-oil or fermentation broth) by using physical techniques as well as the so-called reactive extraction. In reactive (chemical) extraction, a basic compound—*i.e.*, an amine- or organophosphorous-based extractant—is diluted in the organic phase so as to promote the formation of acid–base associations as dominating factor in the extraction system. In this process, the extraction is conducted at or below room temperature, whereas the back-extraction for recovering acids (and/or extractants) is carried out at high temperature. Although previous authors had presented results on reactive extractions at different temperatures, those articles did not provide a totally satisfactory theoretical explanation of the phenomenon. A thorough and highly detailed study was published in 2004 by Canari and Eyal [39] dealing with the effect of temperature on the extraction of carboxylic acids by using lipophilic amines as extractants. The extraction mechanism for acids with these amine-based extractants was divided into two classes: (i) ion-pair formation and (ii) hydrogen bonding and solvation. The ion-pair formation is the dominant mechanism when the amine extractant has a basicity greater than that of the conjugate anion of the analyte. The authors based the study on previous measurements of amine's apparent basicity [40], *i.e.* the $^{s}_{s}pK_a$ of the extractant, as the pH of half neutralization. In contrast, in examples where the pK_a of the amine was lower than the pK_a of the carboxylic acid, extraction was effected principally by either hydrogen bonding or other solvation interactions. In this latter situation, the degree of extraction is mainly determined by the concentration of the molecular fraction of the acid and thus is strongly dependent on the acid's pK_a value. These situations showed stronger temperature effects than those in which ion-pair formation was prevalent.

More recently, Keshav et al. investigated the reactive extraction of propionic acid, a carboxylic acid commonly used in numerous manufacturing processes, with 40% tri-n-octylamine (TOA) in methyl-isobutyl ketone. They studied extractions of the acid in the presence of different chemicals at concentrations close to the real ones found in fermentation broth [41]. The aqueous pH and temperature, among other variables such as different salts, were examined to find conditions that would increase the efficiency of recovery. As expected, and in agreement with previous studies, the authors found a strong effect of pH, with an abrupt decrease in extraction efficiency and no extraction at all at pH values higher than the aqueous pK_a; whereas temperatures between 32 and 40 °C had a negligible effect on the efficiency of the extraction.

Datta and Kuma studied the recovery of formic acid from aqueous solutions using six polar and nonpolar diluents with and without TOA [42]. The effect of temperature was evaluated between 25 and 70 °C, and a sharp decrease in extraction was noted as the temperature increased. The effect of temperature was attributed to the slight increase in the formic acid pK_a, the acid–amine interaction, the solubility changes of the acid in both phases, and the extractant's basicity and water coextraction. This study, however, was conducted without the use of a buffer in the aqueous phase. Similarly, Raheja and Keshav [43] recently showed a significant temperature effect on the extraction of citric acid with TOA in 2-octanol within the range of 30–80 °C from an aqueous non-buffered medium. They observed a 23% decrease in the association constant with respect to the initial value as the temperature became increased by 50 °C.

The supported liquid membrane (SLM) extraction technique, both in flat sheet and hollow fibers, is another well-known variant of LLE extraction for sample preparation [44]. The extraction process involves a partitioning of the analyte from the sample into the organic liquid impregnated in the membrane, followed by diffusion through the membrane towards the acceptor side, ionization and diffusion into the bulk of the acceptor solution (*i.e.*, a back extraction). Therefore, the analyte must be uncharged in order to be dissolved in the membrane, and the pH of the donor solution has to be adjusted in accordance with the pK_a of the compound. The opposite pH condition applies for the acceptor solution. A few publications have reported studies on the influence of temperature in SLM extraction, although they were focused on the influence of temperature on the diffusion coefficient and transport of analytes from the solution toward the supported liquid in the membrane. The first report on those studies described the extraction of nitrophenols from an acid-controlled donor phase through the membrane followed by back extraction into a basic solution [45]. More recently, the extraction efficiency of triazole fungicides at temperatures between 5 and 40 °C was reported [46,47]. Here the pH of the donor solution was controlled by a phosphate buffer of pH 9, and the acceptor solution was HCl. The efficiency results indicated that no noticeable increase was detected when a stagnant acceptor phase was used, but an increase in diffusion occurred when both the donor and the acceptor phases were flowing. In these reports, both equilibria, dissociation of the triazole fungicides and buffers, were strongly affected by temperature.

4. Liquid chromatography

One of the first hydrodynamic consequences of an increase in the column temperature is a decrease in the eluent viscosity. In addition, the diffusion coefficients of a solute molecule will increase significantly. According to the Wilke-Chang equation [48], diffusion coefficients are directly proportional to the absolute temperature and inversely proportional to the fluid viscosity. Hence, for a given column (of fixed length and bed permeability), two possibilities can be envisaged: (i) since the optimum velocity is proportional to the solute diffusion coefficients, the flow rate can be increased with the maximum theoretical plate number remaining constant; (ii) otherwise, for fast chromatography (velocities much higher than the optimum) the column performance deteriorates but less markedly owing to the enhancement in kinetics and in mass transport. The hydrodynamic advantages of working at high temperature have been thoroughly discussed by different authors [49–53]. Therefore, though these specific issues will be taken into account, they are beyond of the scope of the present review.

On the other hand, temperature has a strong effect on the thermodynamics of the retention process, affecting retention factors and, as a consequence, the total analysis time. The influence of temperature on selectivity, however, has been more controversial. Very significant changes have been reported for: i. polymers and oligomers [51,54]; ii. situations under which temperature modifies the stationary phase or the shape and size of both analytes [55] and, iii. ionizable analytes. However, less significant dependences between selectivity and temperature are observed for neutral, low-molecular-weight molecules [56]. Snyder and coworkers have published a series of studies about the simultaneous use of temperature and gradient time, or temperature and elution strength to successfully optimize separation of complex samples using the aid of DryLab software [57–60]. A review published by Dolan has been dedicated to the topic [61].

Many of the studies dealing with the effect of temperature in reversed-phase liquid chromatography (RPLC) had been focused mainly on elucidating the retention mechanism by obtaining thermodynamic parameters from van't Hoff plots. The dependence of retention factors on temperature is given by:

$$\ln k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \beta \tag{29}$$

where ΔH^0 and ΔS^0 are the changes in enthalpy and entropy for the transfer of a solute from the mobile to the stationary phase and β is the column phase ratio. For low-molecular-weight neutral solutes, a linear relationship between ln k and $1/T$ is expected. In Eq. (29), the enthalpy and entropy of transfer are not usually considered as a function of temperature; moreover, the phase ratio is also considered to be temperature-independent. Only few studies have been carried out over a wide temperature range so as to increase the chances of detecting nonlinearity in the plots. Chester and Coym [62], in a critical discussion of the constancy of the phase ratio, proposed a method to distinguish if a nonlinear van't Hoff plot is caused by either a change in the enthalpy of transfer or a change in the phase ratio. The approach consisted in plotting the natural logarithm of selectivity factors (instead of ln k) of a homologous series as a function of the reciprocal temperature. Gritti and Guiochon have emphasized obtaining a linear relationship between ln k versus $1/T$ can indeed be the exception and not the rule [63].

A very different scenario would be expected for ionizable compounds, where selectivity changes associated with temperature can be highly significant. Such temperature effects are especially large for polyelectrolytes such as peptides and proteins. This was clearly demonstrated by Chen and Horváth [64], who compared the influence of temperature on the retention of nitrobenzene and lysozyme over temperatures ranging between 40 and 120 °C, and showed that the slopes of the van't Hoff plots were much higher for the protein than for the low-molecular-weight neutral solute. The authors attributed those temperature-associated increases to changes in conformational structures [65]. Previously, Purcell et al. described a considerable variation in selectivity that they explained by a temperature-induced unfolding process during the RPLC separation of peptides [66,67]. Nevertheless, in addition to changes in conformational structures that depend on temperature, the acidic and basic functional groups would be sensitive to temperature and, as a consequence, contribute to mixed retention mechanisms [68].

Another very different situation arises when an acid–base equilibrium is involved as a variable to adjust chromatographic selectivity towards acidic and basic low-molecular-weight compounds, as it is discussed in the next two sections.

4.1. Influence of temperature on RPLC retention and selectivity

The acid–base equilibrium of an analyte affects its retention in a predicted manner. The equilibrium for the partitioning of the two forms of the analyte between the mobile and the stationary phases, *i.e.*, the transfer of both forms of the analyte, HA and A^-, to the stationary phase (ion charge will be omitted) is given by:

$$HA_m \rightleftarrows HA_s \quad K_{HA} = \frac{[HA]_s}{[HA]_m} \quad \Delta G^0_{HA} = -RT \ \ln \ K_{HA} \tag{30}$$

$$A^-_m \rightleftarrows A^-_s \quad K_A = \frac{[A]_s}{[A]_m} \quad \Delta G^0_A = -RT \ \ln \ K_A \tag{31}$$

where K_i and ΔG^0_i represent the equilibrium constant and the corresponding Gibbs free energy for the distribution of species i between the phases at a given temperature T. The overall retention factor for analyte A will be given by:

$$k = \beta \frac{K_{HA} + K_A({}^s_sK_a/{}^s_sa_H)}{1 + ({}^s_sK_a/{}^s_sa_H)} = \frac{k_{HA} + k_A w_A}{1 + w_A} \tag{32}$$

where $k_{HA} = \beta \cdot K_{HA}$ and $k_{A-} = \beta \cdot K_A$, and $w_A = \left(\frac{K_{a,HA}}{K_{a,B}}\right)\left(\frac{m_B}{m_{HB}}\right)$. As before (Eq. (28)), B and HB refer to the buffer conjugate pair. In these expressions, the acidity constant and the proton activity refer to either ${}^s_sK_a$ (and ${}^s_sa_H$) or, alternatively, ${}^s_wK_a$ (and ${}^s_wa_H$) can be used. The additional assumptions are that (i) the ionization reaction occurs only in the mobile phase, (ii) the rate of dissociation in the mobile phase is very high and, (iii) differences in retention resulting from changes in the identity of the buffering system are negligible (no ion-pair formation). By analogy to the partitioning between immiscible phases [*cf.* Eq. (25)], the thermodynamics of transfer from the mobile phase to the stationary phase can be expressed as:

$$\Delta G^0_{HA} = \Delta H^0_{HA} - T\Delta S^0_{HA} \tag{33}$$

$$\Delta G^0_A = \Delta H^0_A - T\Delta S^0_A \tag{34}$$

A change in temperature will change the transfer of solute from mobile phase to stationary phase as well as the ionization state for both the analyte and the buffer components. A substitution of these thermodynamic expressions, along with values for the thermodynamics of ionization of solute A and buffer B, into Eq. (32) gives

$$k = \beta\left\{\frac{\exp\left[-\Delta H^0_{HA}/RT\right]\exp\left[\Delta S^0_{HA}/R\right] + (m_B/m_{HB})\exp\left[-\left(\Delta H^0_A + \Delta^s_s H^0_a - \Delta^s_s H^0_{a,B}\right)/RT\right]\exp\left[\left(\Delta S^0_A + \Delta^s_s S^0_a - \Delta^s_s S^0_{a,B}\right)/R\right]}{1 + (m_B/m_{HB})\exp\left[\left(\Delta^s_s H^0_a - \Delta^s_s H^0_{a,B}\right)/RT\right]\exp\left[\left(\Delta^s_s S^0_a - \Delta^s_s S^0_{a,B}\right)/R\right]}\right\} \quad (35)$$

Eq. (35) clearly shows that the change in retention factors with temperature might be a very complex function of the operating conditions that will be determined by the enthalpies of transfer of HA and A (usually negative) as well as the enthalpies of the ionization of *both the analyte* and *buffer B*, in addition to the fraction of each of those species present. When $\Delta^s_s H^0_a \neq \Delta^s_s H^0_{a,B}$, the enthalpy of ionization of the selected buffer will strongly affect the dependence of k on temperature. Thus, in a chromatographic system with an eluent pH close to the pK_a of the solutes, the dependence of dissociation on temperature might be used to improve separations involving ionizable solutes by choosing a buffer of the appropriate chemical nature and a temperature within a continuous domain. As Eq. (35) indicates, if the enthalpies of ionization are similar for the selected buffer and the solute, no special effects on selectivity will be expected upon changes in temperature. On the contrary, if the pH of the eluent is close to the solutes pK_a and the two enthalpies of ionization differ significantly, *i.e.*, if $\left|\Delta^s_s H^0_a - \Delta^s_s H^0_{a,B}\right| \gg 0$, then a dependence of selectivity on temperature will be predicted.

The effect of temperature on RPLC selectivity has been controversial, mainly as a consequence of an underestimation of how temperature can influence the dissociation of buffer components and any ionizable analyte and therefore, indirectly, determine retention and selectivity. By following the reasoning depicted in Eq. (32), an analogous overall retention factor for another weak electrolyte, HQ, can be written and the selectivity factor between the two weak electrolytes, A and Q, can be estimated as follows:

$$\alpha_{Q/A} = \frac{k_Q}{k_A} = \frac{\left[K^T_{HQ} + K^T_Q - w_Q(T)\right]/\left[1 + w_Q(T)\right]}{\left[K^T_{HA} + K^T_A - w_A(T)\right]/\left[1 + w_A(T)\right]} \quad (36)$$

This equation should be kept in mind to examine the dependencies of separation factor with the pH of the mobile phase, which will be temperature dependent.

The effect of temperature on retention and selectivity of ionizable solutes under reversed-phase conditions has been pioneered in the seminal work headed by Horváth et al. [54,69,70]. These investigations, conducted in water as mobile phase, demonstrated that the nature of the buffer can significantly modify the selectivity with respect to temperature because of the buffer's enthalpy of ionization. More than a decade elapsed before analysts again focused on the effect of temperature on the RPLC retention of ionogenic solutes, probably due to the fear in the potential damage of the silica-based packings upon heating a column with mobile

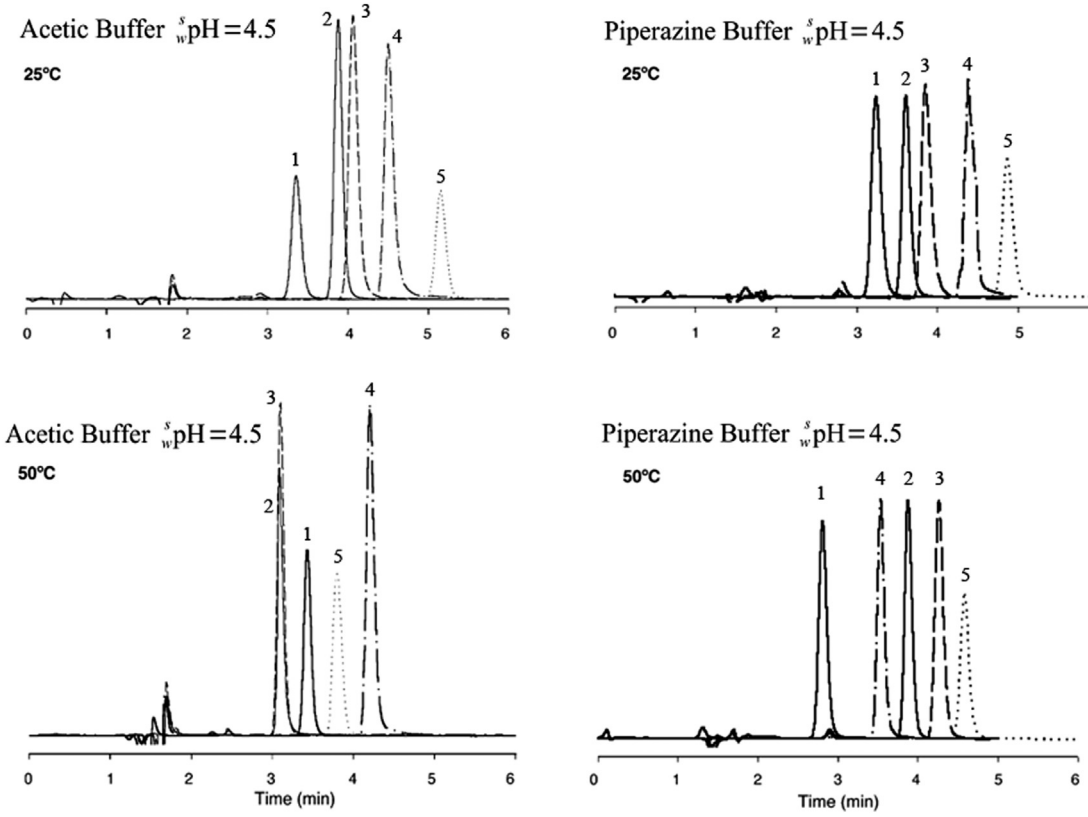

Fig. 5. Chromatograms of five compounds at 25 and 50 °C in two different buffer systems (acetic and piperazine at pH = 4.50). Compounds: 1,4-ethoxyaniline (pK_a = 5.24); 2,2-methylbenzoic acid (pK_a = 3.91); 3,3-bromobenzoic acid (pK_a = 3.80); 4,4-methylaniline (pK_a = 5.08); and 5-cinnamic acid (pK_a = 4.41).

phases containing buffers. In 1996, Snyder et al. [58,59] carried out optimization studies on the retention of a group of solutes that included weak acids and bases, as a function of the mobile phase composition and the temperature. Li published a concise and enlightening theoretical work on the effect of temperature on selectivity in RPLC in situations in which a secondary equilibrium was concurrent [71]. To illustrate these effects, the author calculated the changes in the selectivity resulting from the ionization shift of protonable species as a consequence temperature changes, taking as assumptions the values of aqueous acid–base equilibrium constants, ionization thermodynamic properties and enthalpies of transfer.

McCalley studied qualitatively the retention of three basic solutes at two eluent pHs in a typical RPLC column as a function of temperature ranging from 20 to 60 °C [72] and found that the retention of nortryptyline in a typical RPLC column with mobile phases buffered at pH 7 and of quinine at pH 7 and pH 5 increased with temperature. In 2000, Mao and Carr developed the concept of thermally tuned tandem column (T^3C) [73–75], consisting in the serially coupling of two columns, packed with quite distinct chromatographic materials and independently thermostated, to modify the selectivity continuously by adjusting the individual temperatures of each column. The effect of changing temperature on retention is analogous to the changes in the column lengths, thus altering the relative and absolute contribution of each column to the overall retention factor. The authors described a significant increase in retention factors of basic antihistamines from a hydrophobic octadecylsilica column as the temperature increased that was explained by considering the change in the analyte's degree of ionization as a function of the column temperature [75].

The predictable effect that a change in temperature can have on the retention and selectivity of weak electrolytes using buffers of different chemical nature was assessed in an in-depth study conducted with a typical RPLC column. The study involved different buffers having practically the same ${}^{s}_{w}$pH in 50% methanol/water at 25 °C, but quite different enthalpies of ionization [76]. The retention and selectivity of numerous solutes having pK_as close to the buffer pH demonstrated that negative slopes in van't Hoff plots are possible when the ionization enthalpies of the buffer used to control the pH of the eluents are significantly different from those displayed by the solutes, especially at intermediate degrees of dissociation (pH ≈ pK_a). The studies cited were later extended to acid–base equilibria in 30% (v/v) acetonitrile buffers over temperatures ranging from 20 to 60 °C [77]. As an example, Fig. 5 compares the superimposed chromatograms of five solutes (cinnamic acid, 2-methyl benzoic and 3-bromobenzoic acids, 4-methylaniline and 4-ethoxyaniline) eluted from an octadecylsilica column with mobile phases containing 30% v/v acetonitrile in the buffer acetic acid/sodium acetate ${}^{s}_{w}$pH(25 °C) = 4.95 at 25 and 50 °C (plots on the left) with the same analytes eluted by piperazine/HCl buffer at the same ${}^{s}_{w}$pH and at the same two temperatures (plots on the right). These chromatograms depict the dramatic effect that temperature can have on selectivity in certain particular situations. The solutes chosen have pK_a values close to the pH of the mobile phase, and the increase in temperature had different consequences in each system: the expected decrease in retention was observed when $\Delta\Delta^{s}_{s}H^{0}_{a} = (\Delta^{s}_{s}H^{0}_{a} - \Delta^{s}_{s}H^{0}_{a,B})$ was close to zero (*e.g.*, with acidic solutes run in buffer regulated by acetic acid/acetate or amines run in eluents buffered by piperazine); whereas an increase in retention, or almost no effect at all, is clearly observed when $\Delta\Delta^{s}_{s}H^{0}_{a}$ becomes positive or negative, *i.e.*, when acidic solutes are eluted with a piperazine buffer system, or when amines are eluted with acetic acid/acetate buffer system. From the standpoint of separation, a near baseline resolution of these compounds was obtained with buffer piperazine/HCl at 50 °C taking advantage of temperature.

The effect of temperature on ionizable compounds within a temperature range of 100 °C (*e.g.*, from 30 to 130 °C) was also examined by Heinisch et al. in 2006 [78]. They proposed a chromatographic pK_a, p$K_{a,chrom}$, as the difference

$$pK_{a,chrom} = {}^{s}_{s}pK_{a,T,solute} - \left({}^{s}_{s}pK_{a,buffer} - {}^{w}_{w}pK_{a,buffer}\right) \quad (37)$$

expression which depends on solvent composition, buffer type, and temperature. The introduction of this parameter into Eq. (32) leads to a sigmoidal equation of retention factor as a function of the ${}^{w}_{w}$pH measured at room temperature. The p$K_{a,chrom}$ is obtained under each chromatographic condition by fitting the solute retention factors eluted from a given column at three mobile phase pHs. Even though the proposed strategy would appear quite simple, many requirements limit this approach. For instance, the p$K_{a,chrom}$ is obtained from fitting using a single buffer to keep the shift between ${}^{w}_{w}$pH and ${}^{s}_{s}$pH constant; and this fitting parameter, p$K_{a,chrom}$, has to be obtained for each solvent composition and each temperature. The authors showed several examples of curvilinear plots of ln k against $1/T$ for basic compounds eluted from either a porous graphitic carbon-based or a silica-based column with buffer phosphate in acetonitrile. Fig. 6 is an example of the retentive behavior of four amines (diphenhydramine, chloroprocaine, quinine, and codeine) whose ${}^{w}_{w}pK_a$ ~9 and phenol (${}^{w}_{w}pK_a$~10) eluted with ${}^{w}_{w}$pH = 6.2 phosphate buffer in acetonitrile 30% (v/v) at room temperature. In the presence of 30% acetonitrile, the true pK_as of the amines are not affected significantly (${}^{s}_{s}pK_a$~9), whereas for phenol ${}^{s}_{s}pK_a$~12. The difference between pK_{a2} of phosphoric acid in water and the value in 30% acetonitrile is *c.a.* +0.8 logarithmic units (${}^{s}_{s}$pH was about 7) and $\Delta^{s}_{s}H^{0}_{a}$ = 1.5 kJ/mol [26], which indicates that no significant changes in pH with temperature should occur, whereas a large decrease in ${}^{s}_{s}pK_a$ would be expected for tertiary amines ($\Delta^{s}_{s}H^{0}_{a}$ ≈ 45–50 kJ/mol). Thus, the retention increased as a result of the increase in the fraction of nonionized form of these molecules, *i.e.* $\Delta\Delta^{s}_{s}H^{0}_{a}$ >> 0. The authors proposed a model to

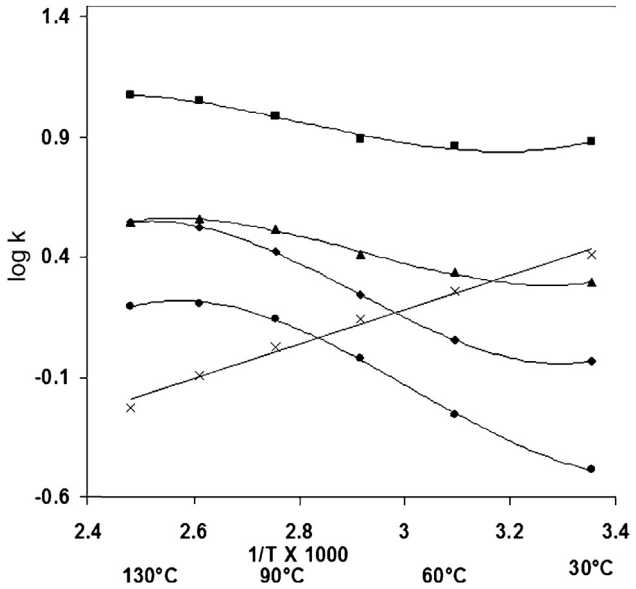

Fig. 6. Variation of the experimental log k vs. $1/T$ for different solutes on a Nucleodur gravity C18 column: diphenhydramine (■), chloroprocaine (◆), quinine (▲), codeine (●), phenol (×). Mobile phase: 70–30 (v/v) 15 mM phosphate buffer at pH 6.2–acetonitrile. Taken from Ref. [78].

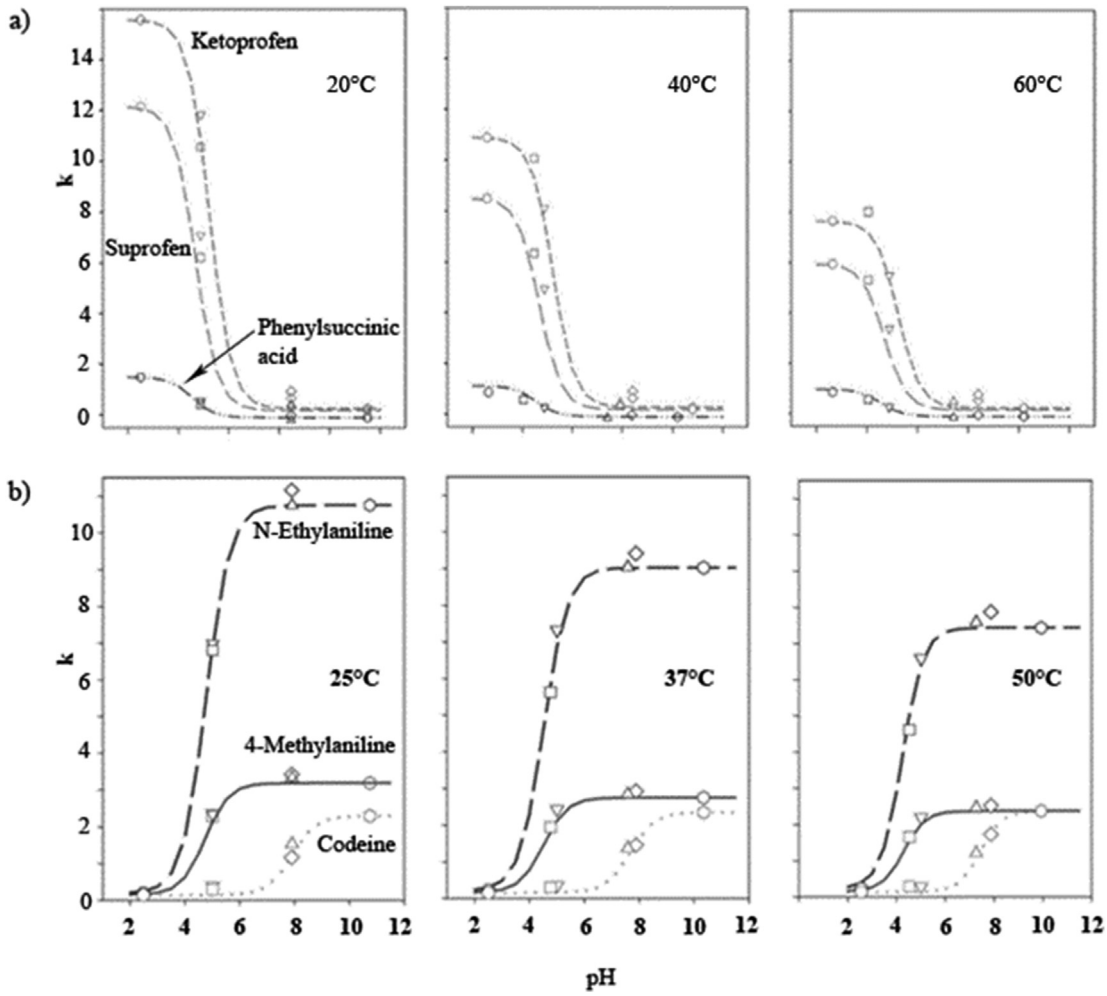

Fig. 7. Predicted and experimental retention factors of: a) three carboxylic acids, as a function of pH at 20, 40, and 50 °C. Mobile phase: buffer solutions in 25% w/w acetonitrile/water, mixture, and b) four amines as a function of pH at 25, 37, and 50 °C. Mobile phase: buffer solutions in 50% w/w methanol/water mixture. Symbols: (●) phosphoric acid/dihydrogen phosphate; (▼) acetic acid/sodium acetate; (■) piperazine/hydrochloric acid; (◆) dihydrogen phosphate/disodium phosphate; (▲) tris/tris:HCl; (□) butylamine/HCl. Column: MS X-Terra C18 (150 × 4.6 mm i.d.). Adapted from Refs. [80,81].

describe retention as a function of mobile phase composition and temperature at a given ${}^{w}_{w}$pH at room temperature.

The retention of azithromycin, chemically a basic compound with ${}^{w}_{w}pK_a = 8.7$, was chromatographed on a PGC column in the range between 30 and 90 °C with mobile phases having different proportions of formic acid and triethylamine and, different ${}^{s}_{w}$pH. The authors attributed the irregular van't Hoff plots to different degrees of ionization for this amine as the temperature increased [79].

Gagliardi et al. deduced a very simple model for the description of the retention behavior of different groups of ionizable compounds comprising amines, carboxylic acids, and phenols on a hydrophobic C_{18} column and with methanol/water and acetonitrile/water mixtures covering a wide range of eluent ${}^{s}_{s}$pH s and column temperatures [80,81]. The equation proposed was derived from the combination of Eqs. (33) and (34) along with Eq. (32), and proved to allow the accurate prediction of the change in retention factors at any temperature and eluent ${}^{s}_{s}$pH, from values at a given reference temperature, T_r:

$$k(T) = \frac{k_{HA}(T_r)\Delta k_{HA} + k_A(T_r)\Delta k_A w(T_r)\Delta w}{1 + w(T_r)\Delta w} \quad (38)$$

where $k_{HA}(T_r)$ and $k_A(T_r)$ correspond to the retention factors of the solute at T_r and at two extreme ${}^{s}_{s}$pH s where α approaches to either 1 (k_{HA}) or 0 (k_A),. The terms Δk_{HA} and Δk_A summarized the exponential term involving the standard enthalpy of transfer, and are calculated, respectively, from linear van't Hoff plots as:

$$\Delta k_{HA} = \exp\left\{\frac{-\Delta H^0_{HA}}{R}\left[\frac{1}{T} - \frac{1}{T_r}\right]\right\} \quad (39)$$

$$\Delta k_A = \exp\left\{\frac{-\Delta H^0_{A}}{R}\left[\frac{1}{T} - \frac{1}{T_r}\right]\right\} \quad (40)$$

and

$$\Delta w = \exp\left\{\frac{\left(-\Delta H^0_{a,B} - \Delta H^0_{a}\right)}{R}\left[\frac{1}{T} - \frac{1}{T_r}\right]\right\} \quad (41)$$

As T equals T_r, Eq. (38) reduces to the sigmoidal function given by Eq. (32). Any change in the column temperature will affect the three terms: Δk_{HA}, Δk_A and Δw. In these expressions, Δk_{HA} and Δk_A are measurements of the sensitivity of the limiting retention factors to temperature changes. For typical RPLC conditions, and considering

that a unique partition retention mechanism takes place, both enthalpies of transfer would be negative. Δw, for its part, depends on the magnitude of the influence that the temperature has on the acid–base equilibrium of buffer and analyte. Thus, any change in temperature would shift the eluent ${}^{s}_{s}$pH according to the sign and absolute value of $\Delta H^0_{a,B}$. In turn, ΔH^0_a will dictate the shift in the solute acid–base equilibrium resulting from changes in temperature. As a consequence of these two combined effects, the Δw value would be either smaller or larger than one, and becomes one when both dissociation enthalpies are equal.

Eq. (38) accurately describes the retention of ionizable compounds based on only four exploratory runs: the experimental measurements of the two limiting retention factors at two temperatures along with the knowledge of the dissociation enthalpies of the buffer used and of the compounds of interest. Fig. 7 shows plots of retention predicted by the model (lines) and experimental data points (symbols) for a number of acids and bases as a function of ${}^{s}_{s}$pH and temperature.

A number of empirical correlations have been used extensively in RPLC to interpret the retention characteristics of neutral compounds, all related to the mobile and stationary phase properties. Those thermodynamic and extra-thermodynamic models are beyond the scope of this review, but excellent discussions based on thermodynamic results [82,83] and comprehensive reviews on extra-thermodynamic models [84,85] are available in the literature. One very useful linear relationship, for a neutral solute and stationary phase, relates the retention factor ($\log k$) to the polarity of the mobile phase as expressed through the Dimroth-Reichardt solvatochromic parameter $E_T(30)$ [86]:

$$\log k = \log k_0 + pE_T(30) \tag{42}$$

where $\log k_0$ and p are constants, dependent on the solute properties. The originally proposed polarity parameter [87,88] was discussed by Cheong and Carr who concluded that this linear relationship, based on a single parameter, was valid for a limited range of solvent compositions [89]. Bosch and Rosés found much better predictions by fitting the retention factors to a normalized solvatochromic parameter P^N_M in replacement of the original $E_T(30)$, as a single parameter representative of the mobile phase polarity [90,91]. This new polarity parameter is related to the solvent compositions by a function that is linear for some solvent compositions but hyperbolic for a wider composition range [92–94]. Subsequently, these empirical equations have shown very good predictive performance used in combination with different forms of Eq. (32) in models applied to ionizable compounds under isothermal conditions [95].

The simultaneous effect of pH, mobile phase polarity and column temperature on the retention of weak acidic solutes has been evaluated by Pous-Torres et al. in three sequential publications [96–98]. In the first, they proposed a model able to predict retention as a function of the three variables starting from the protonation equilibrum (*i.e.* from $1/K_a$). The equation for the retention factor according to this model is:

$$\log k(\varphi, T, \mathrm{pH}) = C_1 + (C_2/T) + C_3 P^N_M + \log\left(C_4 + \left\{\frac{[H^+]10^{C_5+C_6\varphi+(C_7/T)+(C_8\varphi/T)}}{1+[H^+]10^{C_5+C_6\varphi+C_7/T+(C_8\varphi)/T}}\right\}(1-C_4)\right) \tag{43}$$

where the set of C_1–C_8 fitting parameters correspond to the retention of a single solute, φ is the volume fraction, P^N_M is the normalized mobile-phase-polarity parameter, whose value also depends on the volume fraction, and $[H^+]$ denotes the hydrogen ion concentration in pure water. The authors tested the equation with eleven ionizable substances in mobile phases with acetonitrile/buffer. The solution was buffered with citric acid and its salts—*i.e.*, the ${}^{s}_{w}$pH was varied in the range of 3–7 by means of a unique system with acid–base properties (pK_{a1}, pK_{a2} and pK_{a3}) practically insensitive to temperature [98]— within the whole experimental domain. The acetonitrile content was varied between 25 and 45% (v/v) and temperature within the range of 20–50 °C. The degree of fitting was excellent with a root mean square error for the prediction of k-values of 0.45 for 322 k data points ($R = 0.9994$). Naturally, in this system no differences associated with the nature of the buffer components were present. In subsequent studies, the model was used to optimize the separation of mixtures of diuretics and β-blockers, by applying an optimization strategy based on the concept of peak purity as an objective function of the resolution expectancy. The advantage of using this model over polynomial models is that the latter require a larger number of parameters to get similar accuracy, and with the risk of data overfitting. The authors admitted that pH is notably more difficult to model than solvent composition and temperature in order to get acceptable predictions. The robustness of the resulting predictive model was assessed in the last study of the series by using Monte Carlo simulations to estimate the uncertainties in the predictions of retention for peaks in the simulated chromatogram.

Agrafiotou et al. [99] conducted an extensive study in order to include all the potential variables for modeling retention as a function of pH within a wide range; acetonitrile composition and changes in temperature. Thus, the retention factors of twenty-two ionizable solutes were measured at pHs between 2 and 12, in twelve different buffer solutions whose ${}^{s}_{w}$pH was measured at three acetonitrile compositions (20, 40 and 60% (v/v)) and at three temperatures (25, 40 and 55 °C). Each k-pH pair of data was fitted first to Eq. (32) to estimate $k_{HA}(x,T)$, $k_A(x,T)$ and ${}^{s}_{w}pK_a(x,T)$, where the symbol x represents either the volume fraction (φ) or the mobile-phase-polarity parameter (P^N_M). Then the linearity between these fitted parameters and both the mobile phase composition (and polarity) and 1/T were examined. Based on the linear patterns, a general equation that related retention and pH, solvent polarity and temperature was deduced:

$$k_{(\mathrm{pH},x,T)} = \frac{10^{\left(A_1+\frac{A_2}{T}+B_1x+B_2\frac{x}{T}\right)} + 10^{\left(C_1+\frac{C_2}{T}+D_1x+D_2\frac{x}{T}\right)} + 10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}}{1+10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}} \tag{44}$$

In this expression, A_i, B_i, C_i, D_i, E_i, and F_i represent the fitting parameters obtained from experimental k values with all three

significant variables, pH, x and T. The extensive data base included in this study revealed that four of the fitting parameters (those coefficients from the fitting of net charged forms of the solutes) were statistically equal to zero and only eight fitting parameters were strictly necessary. Acids and phenols were fitted to a modified equation that did not include the parameters C_2, D_1, D_2 and E_2:

$$k_{(\mathrm{pH},x,T)} = \frac{10^{\left(A_1+\frac{A_2}{T}+B_1x+B_2\frac{x}{T}\right)} + 10^{(C_1)} + 10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}}{1+10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}} \quad (45)$$

Likewise, amines were fitted to an equation without the parameters A_2, B_1, B_2 and E_2:

$$k_{(\mathrm{pH},x,T)} = \frac{10^{(A_1)} + 10^{\left(C_1+\frac{C_2}{T}+D_1x+D_2\frac{x}{T}\right)} + 10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}}{1+10^{\left({}^{s}_{w}\mathrm{pH}-E_1+\frac{E_2}{T}+F_1x+F_2\frac{x}{T}\right)}} \quad (46)$$

This approach provided excellent results with the advantage of modeling the effects of modifier concentrations, mobile phase ${}^{s}_{w}$pH from 2 to 12, and temperature through the application of a single equation based on only eight fitting parameters. The calculated standard error between experimental and predicted retention factors was 0.622 for 2376 k data points.

Other more recent studies reported the retention of acids and bases in columns of reversed-phase characteristics using eluents containing a buffer solution within a range of temperatures. The effects of eluent pH, temperature, and buffer type and concentration on the selectivity of basic solutes were evaluated on four stationary phases prepared by immobilization of poly(methyloctylsiloxane) on a surface of silica by thermal treatment [100]. No regular trend in selectivity factors with temperature were found for these solutes, nor for phenolic compounds on a polydentate Blaze C8 silica based column using ammonium acetate in different methanol/water mixtures [101].

In summary, temperature is considered an extremely useful variable for method development, and especially for the analysis of ionizable compounds. Although the influence of temperature on selectivity is widely recognized on a qualitative basis, predictable and reproducible results could also be included in optimization programs by taking into account the ionization enthalpies of the buffers and analytes involved.

4.2. Influence of temperature on HILIC

Hydrophylic interaction chromatography is a complex retention mode involving the partitioning of solutes between a surface layer rich in water and the bulk organic mobile phase, along with adsorption mediated mainly by hydrogen bonding or dipolar interactions and electrostatic interactions [3,4,102–105]. Theoretically, all the interactions involved in the retention mechanisms are more or less sensitive to the ionization state of the sample components, the compounds that constitute the buffer in the mobile phase, and the functional groups present on the stationary phase.

Although many researchers recognized the importance of temperature in improving efficiency and peak symmetries, and even in changing selectivity in HILIC [3,103,106,107], very few systematic studies have been published on the use of temperature associated with the retention of ionizable compounds in these systems. Because of the variety of stationary phases that have been examined, different temperature effects have been described in HILIC systems: (i) an increase in the retention at higher temperatures; (ii) the near temperature independence of retention and (iii) systems where the van't Hoff plots are nonlinear. The explanations given for all these situations are varied, and the concurrent effects of temperature on the acid–base equilibria of buffer components and samples are likewise not often recognized.

In 2007, Dong and Huang found temperature effects on the retention of a group of basic antibiotics [108]. For the compounds examined in an organic-rich environment, the temperature response varied with the type of organic solvent used (acetonitrile or methanol); and in addition, different changes in retention with temperature were observed at various pHs, leading to dramatic changes in selectivity observed at higher pH values owing to a temperature-dependent shift in the pK_a.

A thorough review was written by Hao et al. [103] who discussed several positive apparent enthalpies (*i.e.*, negative van't Hoff slopes) for basic and acidic analytes and compared the changes in retention for columns of different chemical properties. They proposed, as an explanation, that the increase in retention as the temperature increased could result from electrostatic interactions with charged silanol groups from the silica-based stationary phases.

Similarly, Kumar et al. [109] investigated the effect of changing the column temperature from 30 to 50 °C, at two compositions of acetonitrile and at a fixed ${}^{w}_{w}$pH of 3.0, in six different HILIC columns with the aim to provide a practical guide to the adjustment of those variables that would influence the selectivity. To simplify the data interpretation, the authors proposed the use of ${}^{w}_{w}$pH rather than ${}^{s}_{w}$pH reasoning that the aqueous pH may be more relevant in retention processes that occur at the water-rich layer adjacent to the stationary phase surface. Neutral and acidic compounds exhibited a typical decrease in retention as temperature increased; but some basic compounds, particularly nortriptyline, procainamide and diphenhydramine, evidenced an increased retention with temperature from a moderate degree up to about 16% on a bare silica column. Nevertheless, the k–k plots of retention at 30 °C versus those at 50 °C exhibited satisfactory correlations, indicating that no significant changes in selectivity were noted for that group of analytes.

An opposite behavior was reported by Heaton et al., who found a change in the elution order between five ephedrines run on a bare silica bridged-ethylene hybrid HILIC column at temperatures between 25 and 50 °C and with ammonium acetate buffer at ${}^{w}_{w}\mathrm{pH} = 5$ and at two concentrations [110]. The apparent enthalpies were strongly dependent on the buffer concentration. The authors attributed those differences in the thermal behavior to changes in the analyte's solvation with temperature at higher buffer concentrations, a hypothesis that had previously been postulated to explain the retention of nucleoside triphosphates [111]. They, however, admitted that the pK_a of ephedrines changes when organic solvent is added and when the temperature is increased. The retention of nucleoside triphosphates in a ZIC-HILIC column without silanol groups and using ammonium carbonate as buffer at ${}^{w}_{w}\mathrm{pH} = 8.9$ in 70% acetonitrile also displayed an increase in retention between 10 and 40 °C [111], although no change in elution order was recorded within this temperature range. It was hypothesized that even when the buffer pH was changing with temperature, that shift was far from the pK_a of the phosphates, thus resulting in no changes in the analyte's ionization status.

An increased retention of aspirin on an amine-based column within the temperature range between 20 and 70 °C was reported by Guo and Gaiki in 2005 [112]. Positive apparent enthalpies and nonlinear van't Hoff plots were also observed for weak electrolytes in different zirconia-based columns run with a fixed buffer

composition (ammonium acetate at ${}^{w}_{w}pH = 4.5$ in 85% (v/v) acetonitrile), within a wide temperature range [107,113]. Similar trends were found when twelve out of fifteen ionizable solutes were chromatographed onto zirconia or on hybrid magnesia-zirconia stationary phases at temperatures between 15 and 55 °C, although the mobile phase pH was not specified [114].

Chirita et al. studied the retention of twelve ionizable compounds in two zwitterionic HILIC columns within the range of 10–50 °C with a mobile phase comprised of 80% (v/v) acetonitrile and 20% ammonium acetate buffer at ${}^{w}_{w}pH = 4$ (${}^{s}_{w}pH = 6.2$) and observed nonlinear plots with different trends (the retention of six bases increased whereas it decreased for six acids and amino acids as temperature increased) [115]. The authors recognized that nonlinear plots were consistent with the concurrent dissociation properties of the solutes. Similarly, nonlinear plots for six pyrimidines and six purines eluted from a ZIC-HILIC column with a mobile phase containing 1 mM ammonium acetate at ${}^{w}_{w}pH = 4$ in acetonitrile were observed whereas the same analytes exhibited linear plots on an amide-based column [116].

Qiu et al. [117] conducted thermodynamic measurements of fifteen solutes (including several ionizable analytes) chromatographed on a zwitterionic HILIC column in a mobile phase consisting of 90% (v/v) acetonitrile + 10% (v/v) 20 mM ammonium acetate at ${}^{w}_{w}pH = 4.1$, within the temperature range of 10–70 °C. They also noted positive (though practically null) enthalpies and a nonlinearity of van't Hoff plots for some basic drugs, that the authors attributed to a temperature-induced change in the column phase ratio for those solutes. That hypothesis was based on the previous studies conducted by Chester and Coym on RPLC columns with different densities of bonded aliphatic chains [62]. In contrast, linear plots with positive slopes have been described for uracil, uridine, cytosine, cytidine and orotic acid run on a cysteine-based zwitterionic column with ammonium formate at ${}^{w}_{w}pH = 3.25$ mixed with 85% (v/v) acetonitrile at temperatures ranging between 20 and 60 °C [118], for nucleic acids and nucleosides on a cyclofructose-based column with 90/10 (v/v) acetonitrile/20 mM ammonium acetate at ${}^{w}_{w}pH = 4.1$ [119], and for nine ionizable solutes (acids and amines) on an imidazolium-based zwitterionic stationary phases with 90/10 (v/v) acetonitrile/10 mM ammonium acetate between 25 and 50 °C [120].

Despite of all the studies reviewed, most having been published in recent years, we are not aware of a single thorough and systematic investigation that included several buffered mobile phases and ionizable analytes on HILIC columns covering a range of temperatures. The growing interest in this chromatographic mode, potentially extremely useful for polar, weakly acidic or basic compounds, would be a strong motivation for undertaking this task.

5. Electrophoretic techniques

In CE, temperature studies have been addressed, in most instances, to the heat generated by the circulation of a current through a resistance, the so-called Joule heating. This effect in CE was studied early by Hjertén [121] and in the decades following by many other researchers [122–126]. Owing to only partially efficient heat dissipation, radial gradients of temperature develop with the highest temperature resulting in the capillary center. This radial thermal profile produces several negative effects on electrophoretic separations [127–129], as will be discussed in detail. On the other hand, temperature can be used as an effective tool for optimizing electrophoretic separations [130,131].

Several individual studies were carried out to investigate the consequences of changing the physical properties of the separation medium during electrophoretic processes. The well-known effect of temperature on viscosity [122,132–134], dielectric constants [135–137] and diffusion coefficients [48,138–140] is worth mentioning. Despite of this, the effect of deliberately controlling temperature externally on electrophoretic processes has been scarcely studied, and only few authors have proposed theoretical contexts to describe the behaviors of each operational variable as a function of temperature. Gaš [141] presented a theoretical background for the establishment of an axial profile of temperature and a description of the interactions at the temperature interfaces. Evenhuis et al. [142] made a systematical study of the zeta potential as a function of temperature.

More recently, Rogacs and Santiago [143] reviewed the most relevant effects of temperature within the whole CE system, employing fundamental physical equations to describe the behavior of the actual mobility. In addition, to describe the variation in the acid dissociation constants with temperature, the authors stated both solutions of the van't Hoff differential equations (Eq. (5) and (6)). Therefore, upon consideration of the thermodynamic data available in the literature, a prediction of the constants at different temperatures could be performed with great accuracy [143].

5.1. Electrophoretic mobility and selectivity

The changes in capillary temperature strongly affect several physical and chemical operational variables, which in turn have a great impact on the electroosmotic and electrophoretic mobilities. A few studies have reported the use of temperature as a tool for improving the selectivity of the electrophoretic separations without an established theoretical background—that is, by trial and error. In most of the circumstances, this approach assessed variations in affinity constants in order to separate or to evaluate the interactions between biomolecules or chiral compounds [144–147], while other authors applied this operational variable to either denature or achieve conformational changes in proteins [148,149].

The electroosmotic mobility (μ_{eo}) for an open capillary tube is defined as the quotient between the electroosmotic velocity (v_{eo}) and the applied electric field (E) as follows,

$$\mu_{eo} = v_{eo}/E = (\varepsilon_0 \varepsilon_r \xi)/\eta \quad (47)$$

where ε_0, ε_r, ξ and η denote the vacuum permittivity, the dielectric constant, the zeta potential, and the viscosity of the background electrolyte (BGE), respectively. The viscosity and, to a much lesser extent, also the zeta potential and the dielectric constant depend on temperature. Although early studies proposed that the product $\varepsilon_r \xi$ remains constant against changes in temperature [122], recent work has demonstrated that the occurrence of individual variations results in a decrease in the product of about 0.07% per degree (°C). Thus, in practice, this value could be ignored in comparison to the sensitive variation in the viscosity, around a 2.28% of increment per degree (°C) [142]. Hence, the increment of capillary temperature will increase the magnitude of the electroosmotic flow (EOF) and thus significantly decrease the analysis time. In addition, in the case of cations and positive mode, a decrease in the residence time in the capillary column should improve the peak efficiency, though at the same time an increment in temperature increases the diffusion coefficient and, as a consequence, promote band broadening [150]. As a result, the net change in the peak efficiency with temperature becomes a trade-off between the aforementioned phenomena.

The apparent mobility of an acidic monoprotic analyte A ($\mu_{app,A}$) in the presence of electroosmotic flow is,

$$\mu_{app,A} = \mu_{eff,A} + \mu_{eo} \quad (48)$$

where $\mu_{eff,A}$ is the effective mobility of the analyte *A*, defined as,

$$\mu_{eff,A} = \mu_{act,A}\alpha \quad (49a)$$

or

$$\mu_{eff,A} = \mu_{act,A}(1-\alpha) \quad (49b)$$

which expression is the product of the actual mobility, $\mu_{act,A}$, and the degree of ionization for either protonated bases (α) [Eq. (49a)] or neutral acids (1- α) [Eq. (49b)]. The actual mobility is given by:

$$\mu_{act,A} = \frac{q}{6\pi r \eta} \quad (50)$$

where q is the analyte ionic charge and r its hydrodynamic radius. The actual mobility and the EOF depend on temperature in the same manner, because both magnitudes are inversely proportional to the dynamic viscosity. This dependency results in increments in mobility with temperature without changes in selectivity between the analytes. On the other hand, the degree of ionization can depend on temperature in a more complex way, as deduced from Eq. (23). Other mathematical expressions of α extended to polyprotic ionizable compounds have been developed [151].

Introducing the degree of ionization into Eq. (49) leads to an expression of effective mobility that is a function of the pH of the BGE and the pK_a of the ionizable compound.

$$\mu_{eff,A} = \frac{\mu_{act,A}}{1+w_A} = \frac{\mu_{act,A}}{1+10^{z(pH-pKa)}} \quad (51)$$

where z = 1 for cations and z = −1 for anions. As in previous sections, pK_a and pH values correspond to a constant solvent composition and temperature, usually water ($^{w}_{w}$pH and $^{w}_{w}pK_a$). With the standard enthalpies, entropies and activity coefficients being considered invariants in the studied range of temperatures, the variation in the equilibrium constant could be described by a linear van't Hoff equation. Thus, from the thermodynamics of the ionization of solute A and buffer B, described in Eq. (20), the following expression can be obtained:

$$\mu_{eff,A} = \frac{\mu_{act,A}}{1+\left(\frac{m_B}{m_{HB}}\right)\times \exp\left[\frac{-(\Delta H^0_a - \Delta H^0_{a,B})}{RT}\right]\times \exp\left[\frac{(\Delta S^0_a - \Delta S^0_{a,B})}{R}\right]} \quad (52)$$

This expression proves to be very descriptive of how the analytes behave when the capillary temperature changes and consequently can be used to predict the variation in their electrophoretic mobilities. The effect of temperature on the degree of ionization might lead to three different situations depending on the values of enthalpy of ionization of both the BGE ($\Delta H^0_{a,B}$) and the analyte (ΔH^0_a): (i) $\Delta H^0_{a,B} > \Delta H^0_a$, a change in pH results from a shift in BGE pK_a, and thus in the mobility of the ionizable compound; (ii) $\Delta H^0_{a,B} < \Delta H^0_a$, the pH of the BGE remains practically constant as the temperature changes, but the pK_a of the analyte undergoes a modification thus altering the distribution coefficient and consequently the mobility; (iii) $\Delta H^0_{a,B} \approx \Delta H^0_a$, *i.e.*, $|\Delta H^0_{a,B}\ \Delta H^0_a \approx 0$, the difference between the ionization of both the BGE and the analyte remains unalterable with temperature changes. As an example, Fig. 8 shows the electropherograms of 2,3,5-trimethylpyridine and 2,4,6-trimethylpyridine at different temperatures, with two different BGEs (unpublished results from our laboratory). The times were normalized by an EOF marker, taking 2,3,5-trimethylpyridine as a reference, in order to avoid differences in the analysis time caused by the variations in the viscosity, the zeta potential and the dielectric constant of the media. Electropherograms shown in plot 8a) were obtained with a BGE constituted by a piperazine/HCl buffer regulated at $^{w}_{w}$pH = 4.80 (at 25 °C). Since the enthalpy of ionization of the BGE was 30 kJ/mol, the pK_a decreased and thus, the pH dropped by about 0.65 pH units between 25 and 60 °C. Furthermore, the enthalpies of dissociation of both analytes

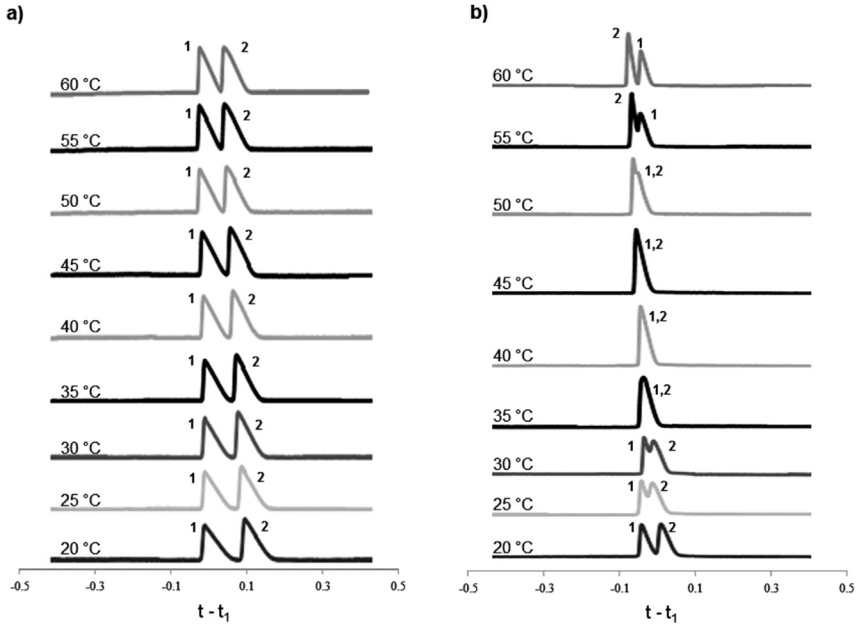

Fig. 8. Separation of 2,3,5-trimethylpyridine (1) and 2,4,6-trimethylpyridine (2) at different temperatures between 20 and 60 °C. (a) BGE is piperazine:HCl at $^{w}_{w}$pH = 4.80 and 25 °C and, (b) BGE is acetic acid/acetate at = 4.80 $^{w}_{w}$pH and 25 °C. Capillary dimensions: L_t = 60 cm, L_d = 52 cm and id = 75 μm. Separation voltage: 20 KV and detection wavelength at 254 nm. The variable t is the time corrected by an EOF marker and t_1 is the reference time associated to analyte 1.

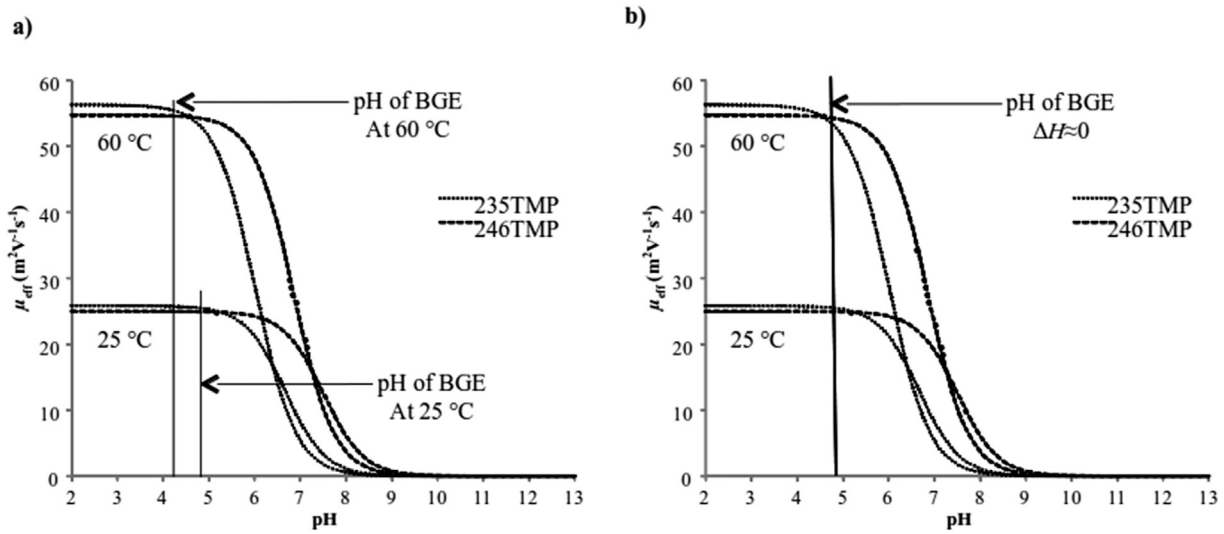

Fig. 9. Plots of effective mobility as a function of pH at 25 and 60 °C for 2,3,5-trimethylpyridine (235TMP) and 2,4,6-trimethylpyridine (236TMP). (a) BGE is piperazine:HCl at ${}^{w}_{w}$pH = 4.80 and 25 °C and, (b) BGE is acetic acid/acetate at ${}^{w}_{w}$pH = 4.80 and 25 °C as in Fig. 8.

were similar (*i.e.*, 34.9 kJ/mol for 2,3,6-trimethylpyridine [23] and 32.4 kJ/mol for 2,4,6-trimethylpyridine [99]), so the drop in the pH of the BGE was comparable to the respective decreases in the analyte pK_a; thus producing no significant changes in the selectivity. In contrast, in the electropherograms displayed in Fig. 8b, a BGE based on an acetic acid/acetate buffer at ${}^{w}_{w}$pH = 4.80 (at 25 °C) was used. The enthalpy of dissociation of this carboxylic acid is close to zero, *i.e.* ($\Delta H^0_a - \Delta H^0_{a,B}$) >> 0. As a result, the pH of the separation remains constant within the whole temperature range studied, while the analytes reduce their respective pK_a values by about 0.65 units; thus effecting an inversion in the order of migration. The total loss of selectivity occurs at an intermediate temperature. Fig. 9 depicts the theoretical electrophoretic results describing the sigmoidal trend in effective mobility as a function of pH at 25 and 60 °C for 2,3,5-trimethylpyridine and 2,4,6-trimethylpyridine. Plots (a) and (b) correspond to the two experimental BGEs shown in Fig. 8.

In 2007, Reijenga et al. [131] published a proof-of-principle article describing the impact of the dependence on temperature of the acidity constants and the use of this operational variable to improve the selectivity of a separation in CE. Two common BGEs used in CE were monitored as a function of temperature. The results indicated that whereas a phosphate buffer remained at a practically constant pH within the whole temperature range studied, a histidine buffer decreased the pH from 6.35 at 10 °C to 5.50 at 50 °C. Additionally, this work considered the separation of model analytes, where variations in the pK_a as a function of temperature were also tested to prove the dependence of the selectivity on temperature—all these experiments were performed with predictive purposes.

Based on this previous work, Reijenga [152] studied the effect of temperature gradients as a function of time in order to develop a highly sensitive gradient of pH considering both, the dpK_a/dT and the dpH/dT of different buffers commonly used as BGEs in CE. The separation of eight weak acids was first optimized theoretically and then demonstrated experimentally by using these temperature-induced pH gradients.

5.2. Other applications of temperature in CE

On-line preconcentration is another important issue in CE method development. Only few pioneering studies addressing the use of temperature in electrophoretic preconcentration have been reported in the literature. Ross et al. published a series of papers describing the application of temperature gradients along the capillary to successfully focus and concentrate the analytes either as a single procedure or coupled to other on-line preconcentration techniques [153–156]. Mandaji et al. [157,158] proposed to generate pH interfaces along the capillary induced by temperature changes. This approach would be carried out by taking into account the variations of the pK_a of both, analytes and BGE, as a function of temperature; the analyte diminished its mobility by changing the dissociation degree or by changing the BGE pH and, consequently, it is stacked up at the temperature interface. The best results achieved, though, were only a 2-fold preconcentration in the absence of EOF.

One of the most frequently used CE modes is micellar electrokinetic capillary electrophoresis (MEKC). In this technique, a variation in temperature modifies the distribution coefficient of the analyte between the micelles and the BGE solution caused by the variations in the critical micelle concentration (CMC) and the partial specific volume, in addition to the aforementioned variations associated with the viscosity [159–161]. Changes in the temperature have been employed as a tool to optimize the separations of twenty-three dansylated amino acids by MEKC [160,162].

Temperature was also used in capillary gel electrophoresis to modify the sieving effect of the polymer matrices, besides the increment on the electrophoretic mobility of the analytes. Several research articles have focused on the analysis of DNA molecules with successful results [130,163–165]. Klepárnik et al. extended the read lengths in DNA sequencing by changing the temperature [130]. They reported optimal results at a column temperature of 55 °C, which both reduces the analysis time and improves the resolution of the DNA fragments with high numbers of base pairs. A possible explanation stated by the authors was an increment in the thermal energy of the DNA molecules along with variations in the sieving matrix. Temperature changes were also implemented in the analysis of single-stranded-DNA conformational polymorphisms in the screening of unknown mutations in short stretches of DNA. The best results for various genes were obtained with short-chain linear polyacrylamide as a sieving matrix at temperatures below 20 °C [166,167]. Small temperature gradients were also demonstrated to be useful in making a quick and precise estimation of the existence of a point mutation in an amplified DNA fragment [168].

Very recently, Tascon et al. studied the effect of temperature in on-line solid phase extraction CE [169]. The authors achieved an improvement of about 3-folds in the LODs of a group of peptides by thermostatting the preconcentration cartridge at a temperature of 60 °C.

In conclusion, two approaches can be useful for employing temperature as an operational variable to optimize electrophoretic separations. As stated in most examples from the literature, the optimization of the conditions without a theoretical background requires a systematical study in order to obtain the optimal results. In contrast, to achieve a fast and thorough optimization procedure, the fundamentals of both the technique and the chemical equilibria involved in the separation process must be established.

5.3. Determination of pK_a

Although several works have been reported in the literature related to the determination of thermodynamic dissociation constants in water and at different solvent compositions by CE [24,151,170–175], only a single study performed measurements between 20 and 50 °C by using the so-called internal standard CE (IS-CE) method applied to several compounds [24,176]. Basically, this approach consists in choosing an internal standard with very close conformational and thermodynamic properties to those of the analyte. Thus, to obtain the pK_a of a monoprotic compound, only two electrophoretic runs are needed. In the first, the effective mobilities of both the analyte (test compound) and the IS are measured and, in the second, their limiting mobilities are recorded (*i.e.*, the mobility of the completely charged species). The mobility data of the IS together with its pK_a value are used to calculate the exact pH of the solution inside the capillary, and then the pK_a of the analyte is obtained through the analyte's electrophoretic data [176]. This method offers the advantage over the classical procedure in that an external measurement of the exact pH of the buffer solution is not needed, thus making the IS approach much faster.

6. Practical considerations

6.1. Heat transfer efficiency and thermostatization

Even though temperature offers a variety of possibilities for improving separations, the practical use of thermal effects on dynamic systems involves transfers of heat and temperature gradients, which are associated with profiles of many properties of the physical and chemical environment.

Temperature gradients perpendicular to the separation axes were early associated with profiles of viscosities as the main cause of loss of efficiency. In electrophoresis this issue was initially addressed on early years by Tiselius [177]. Three decades later, quantitative models describing the velocity profiles across the solute band were reported by S. Hjertén [121]. Likewise, in LC, detailed models describing the temperature and viscosity profiles and the band broadenings have been reported by Halász et al. [178]. More recently, different authors have reviewed this issue, improving the early models to represent the profiles and applied the improvements to modern high-performance CE [127] and HPLC systems [179].

Although early papers by Hjertén and Halász mentioned that the temperature can have effects on parameters other than viscosity—*e.g.*, retention factors, pK_a values, or the degree of dissociation—only in the last decade work was published determining experimentally the change in the averaged retention factor as a function of the solvent flow [179,180].

In the absence of a more comprehensive model, we can state that the contribution of any property to the band broadening whose profile is temperature-associated could be diminished by reducing the radial temperature gradient. Poppe et al. [181] listed four interesting approaches to reducing the temperature profiles in LC but also applicable to CE systems: (i) avoid the use of high pressures (or high voltages), (ii) use the concept of infinite diameter, (iii) use insulated (*i.e.*, adiabatic) columns (or tubes), and (iv) improve the radial transfer of heat in order to smooth down the radial profile.

The first two approaches are notable, though those practices can be considered as only a proof of concept and far from being applicable in real situations. Low pressures or low voltages are contradictory to a reduction in analysis time. The concept of infinite diameter consists in the use of a very wide column, injecting the sample into only the central zone where the temperature profile is flat. This implies a separation along the column length using only the central zone of the cross-section. With respect to the use of insulated or adiabatic columns that produce a reproducible temperature profile over the time, the few reports found in the literature evaluated the magnitude of temperature gradients developed as well as the efficiencies achieved by columns packed with sub-2.5 μm particles inside of tubes of narrow internal diameter [179,182,183].

The last approach has been, by far, the most investigated. Two strategies aiming to flatten the radial temperature gradients have been used: (i) improving the radial transfer of heat by reducing the column diameters, from standard diameters toward narrow-bore, micro-bore, and even capillary columns; or (ii) reducing the heat exchange through the column wall by using efficient systems to preheat the mobile phases before entering the thermostatted column.

Despite miniaturization results in an advantage in terms of heat transfer, channels inside of solid matrixes (chips) constitute a regression in the improvement of the heat transfer and thus can be expected to limit experimental efficiencies and even reproducibility.

As regards the use of temperature in packed chromatographic columns, thermostatization of the incoming mobile phase before entering the column has been demonstrated to be effective in reducing radial thermal profiles. Considering that viscous friction will produce an increase in temperature, Poppe et al. demonstrated that the best results in terms of efficiencies can be achieved by thermostatting the incoming mobile phase some degrees below the column temperature [184]. More recently, Yan et al. [185] and also Guillarme et al. [186] made calculations on the proper capillary geometries to get preheated incoming eluents with a minimum extracolumn volume. Recently, different devices for heating the incoming mobile phases, called preheaters, have been introduced. The high pressures used in HPLC allow heating at temperatures much higher than the boiling point at atmospheric pressure. The free elution of such overheated mobile phases into pressure down to atmospheric levels would result in a sudden vaporization immediately after the final restriction. Conversely, an overheated state could prove advantageous for some detectors, though not for others that require an eluent cooler immediately in tandem with the column but before the final drop in pressure. These practical aspects have been thoroughly compiled in a book authored by Teutenberg [187].

As to the thermostatization of capillaries in CE, forced-air convection has been proved to be as good as the systems based on circulation of liquids. A very fundamental and still unsolved issue in CE is that the Joule effect produces heat all along the capillary tube, with the usual set up of the capillary in modern CE instruments being coiled in a cassette or cartridge. Cassettes restrict the possibility of thermostatization to only the central section of the capillary length, thus not achieving a control of temperature at both

ends. Temperature steps between well and poorly thermostatted zones can produce uncontrolled or otherwise undesirable effects such as thermal stacking as discussed previously. Moreover, the nonthermostated length of capillaries varies from one model of commercial instrument to another, thus affecting the separation results in terms of repeatability, reproducibility, and transferability of methods.

6.2. Determination of the pH

In order to succeed in the optimization of analyte separation, the pH must be accurately measured and then accordingly adjusted in the medium used. As a consequence, some precautions must be taken in order to maintain a satisfactory reproducibility. Thus, glass electrodes must be in good condition: (i) the asymmetry potentials (potential measured with an aqueous standard buffer of pH = 7.00) must be ideally zero or, otherwise below 20 mV; and (ii) the difference between the potentials corresponding to buffers of pH = 4 and pH = 7 should be above 165 mV (*i.e.*, response slope above 0.055 V).

Calibrations must be performed strictly following the multipoint procedure recommended by IUPAC in the standardization rules [188,189], using at least three freshly prepared pH standard buffer solutions, whose pH ranges must include the value where the electrode has to be used. Finally, the potentiometric readings must be taken under equilibrium conditions. Since the response speed of glass membranes is usually slower in hydroorganic mixtures than in pure water, a check for slow drifts of pH before setting the calibration value and during the pH readings is strongly recommended.

Because temperature can affect the pK_a and also the pH of calibration buffers, attention must be paid to the proper thermostatization of the solutions. The addition of strong acids or bases to adjust the pH can lead to either endo- or exothermic reactions. Therefore, the final pH value must be obtained at the same temperature.

Nowadays, the scale conversion parameter, δ (Eq. (17)), is available for electrodes with internal salt bridges of 3M KCl to convert ${}^{s}_{w}$pH in methanol/water, acetonitrile/water or ethanol/water mixtures. For high organic-solvent compositions—*i.e.* HILIC mobile phases—these conversion parameters are significantly high and have associated large standard deviations. Other electrodes designed specifically to measure pH in organic solvents, such as those with salt bridges of ethanol saturated in LiCl, could probably lead to more reproducible pH values, but conversion parameters are not available for these electrodes.

6.3. Availability of pK_a in the literature

Accurate pK_a values are needed to predict chromatographic and electrophoretic behaviors or to test the fidelity of a previously proposed approach. The pK_a values are usually searched in the literature, where a wide variety of determination methods and chosen standard states are reported. For LC predictions, clearly, ${}^{s}_{s}pK_a$ (or ${}^{s}_{w}pK_a$) values are needed. Equations to estimate these pK_a values in acetonitrile and methanol mixtures have been proposed [88]. Similarly, the availability of pK_a values is very limited at temperatures far from 25 °C, and thermodynamic enthalpies and heat capacities are likewise not widely determined. A database of more than 900 pK_a values, several at 37 °C has been recently compiled [1].

7. Outlook and perspectives

Since the proton-transfer process is frequently an integral part of the analytical separation practice, this equilibrium cannot be overlooked. Hence, whenever a separation involves weak acidic or basic compounds, the pH should be used as a first trial to undertake separation. Models should be applied to predict retention or electrophoretic mobility of analytes as a function of pH. Sigmoidal dependences with an inflection point corresponding to the solute pK_a have been theoretically deduced. Precisely within this pH range the largest effect on selectivity between two compounds with different pK_a values may be observed. Therefore, utilizing temperature as a means of manipulating the degree of ionization of weak electrolytes in buffered liquid systems can provide a very simple, predictable, reproducible and continuous means of modifying selectivity.

The increasing interest in the potential of HILIC techniques for the analysis of acidic and basic substances suggests that a wealth of information would be gained through systematic and comprehensive studies of multiple HILIC systems that would include representative column chemistries as well as different eluent pHs, solvent compositions, and temperatures. Essentially, an effort intending to understand these complicated separation systems should be considered in future work.

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List of symbols

a_i	activity of the species i (H^+ proton, A^- dissociated ion and HA undissociated acid)
${}^{s}_{w}a_i$; ${}^{s}_{s}a_i$; ${}^{w}_{w}a_i$	activity of the species i in solvent s or in water (superscript) with standard state in the same solvent (s) or water (w) (subscript)
m_i	molality of species i
γ_i	activity coefficient of species i
$\gamma_{t,i}$	primary medium effect for the transfer of the species i from water to the solvent s
G_i	partial molal Gibbs free energy of solute i
${}^{s}_{s}G^0_i$; ${}^{s}_{w}G^0_i$	standard partial molal Gibbs free energy of solute i in a given solvent s (superscript) with standard state in the same solvent (s) or water (w) (subscript)
$\Delta G^0_{t,i}$	change in Gibbs free energy of transfer of one mole of the species i from the standard state in water to the standard state in the organic solvent
$\Delta H^0_{t,i}$	change in enthalpy of transfer of one mole of the species i from the standard state in water to the standard state in the organic solvent
HA, A^-	acidic and basic forms of the analyte
HB, B	acidic and basic forms of the buffer
K_a	thermodynamic acid dissociation constant in water of the analyte, HA
${}^{s}_{s}K_a$; ${}^{s}_{w}K_a$	thermodynamic acid dissociation constant of HA in solvent s (superscript) with standard state in the same solvent (s) or water (w) (subscript)
${}^{s}_{s}K_{a,B}$; ${}^{s}_{w}K_{a,B}$	thermodynamic acid dissociation constants for the buffer in solvent s with standard state in the same solvent (s) or water (w)

ΔH^0_a change in standard enthalpy for dissociation of HA in water

$\Delta C^0_{p,\,a}$ change in standard heat capacity for dissociation of HA in water

Θ reference temperature

$\Delta^s_s G^0_a$; $\Delta^s_w G^0_a$ change in standard Gibbs free energy for the proton transfer equilibrium of HA in the solvent mixture s (superscript) with standard state in the same solvent (s) or water (w) (subscript)

$\Delta^s_s H^0_a$; $\Delta^s_w H^0_a$ change in standard enthalpy for proton transfer equilibrium of HA in the solvent mixture s (superscript) with standard state in the same solvent (s) or water (w) (subscript)

$\Delta^s_s S^0_a$; $\Delta^s_w S^0_a$ change in standard entropy for proton transfer equilibrium of HA in the solvent mixture s in solvent s (superscript) with standard state in the same solvent (s) or water (w) (subscript)

$\Delta^s_s G^0_{a,B}$; $\Delta^s_s H^0_{a,B}\Delta^s_s S^0_{a,B}$ ($\Delta^s_w G^0_{a,B}$; $\Delta^s_w H^0_{a,B}$; $\Delta^s_w S^0_{a,B}$) thermodynamic functions for the dissociation equilibrium of the buffer system

${}^w_w pH$ pH in water

${}^s_w pH$ pH in solvent s in reference to water as standard state. In practice, pH measured in solvent mixture with the electrode system calibrated in aqueous standards

${}^s_s pH$ pH in solvent s in reference to the same solvent as standard state. In practice, pH measured in solvent mixture with the electrode system calibrated with standards in the same solvent

$\overline{E}_j$ residual liquid-junction potential error

δ parameter of correction between both scales

K equilibrium constant for the transfer of HA between two phases

D distribution ratio

$C_{HA\,(o)}$; $C_{HA\,(w)}$ total concentration of HA in organic solvent (o) or in water (w).

m_{HA} total concentration of HA expressed in molal scale.

α fraction between HA and total concentration

ΔG^0_{HA}; ΔG^0_A change in standard free energy for the distribution of HA (and A^-) between phases

ΔH^0_{HA}; ΔH^0_A change of enthalpy for the distribution of HA (and A^-) between phases

ΔS^0_{HA}; Δk_{HA} change of entropy for the distribution of HA (and A^-) between phases

k_{HA} retention factor of the protonated form of the analytes

k_{A-} retention factor of the deprotonated form of the analyte

β column phase ratio ($= V_s/V_m$, where V_s and V_m are the volumes of stationary and mobile phases, respectively)

$\alpha_{Q/A}$ selectivity factor between acidic solutes Q and A

Δk_{HA} variation in k_{HA} between temperatures T and a reference temperature T_r (for a given solvent composition)

Δk_A variation in k_A between temperatures T and a reference temperature T_r (for a given solvent composition)

$E_T(30)$ Dimroth-Reichardt solvatochromic parameter log k_0 intercept of linear variation between log k and $E_T(30)$

p slope for the linear variation between log k and $E_T(30)$ in the polarity parameter model

P^N_M mobile phase polarity parameter in the polarity parameter model

C_1–C_8 fitting parameters of Eq. (44) corresponding to the retention of a single solute at a given pH, volume fraction and temperature

φ volume fraction

A_1 intercept for the linear dependence of log k_{HA} with $1/T$

A_2 slope for the linear dependence of log k_{HA} with $1/T$

B slope for the linear dependence of log k_{HA} with x (volumetric fraction or P^N_M)

B_1 intercept for the linear dependence of B with $1/T$

B_2 slope for the linear dependence of B with $1/T$

C_1 intercept for the linear dependence of log k_A with $1/T$

C_2 slope for the linear dependence of log k_A with $1/T$

D slope for the linear dependence of log k_A with x (volumetric fraction or P^N_M)

D_1 intercept for the linear dependence of D with $1/T$

D_2 slope for the linear dependence of D with $1/T$

A_1 intercept for the linear dependence of pK_a with $1/T$

E_2 slope for the linear dependence of pK_a with $1/T$

F slope for the linear dependence of pK_a with x (volumetric fraction or P^N_M)

F_1 intercept for the linear dependence of F with $1/T$

F_2 slope for the linear dependence of F with $1/T$

μ_{eo} electroosmotic mobility

υ_{eo} electroosmotic velocity

E electric field in electrophoresis method

ε_0 vacuum permittivity

ε_r dielectric constant

ξ zeta potential

η viscosity of the fluid

$\mu_{app,A}$ apparent mobility of A

$\mu_{eff,i}$ effective mobility of the analyte i

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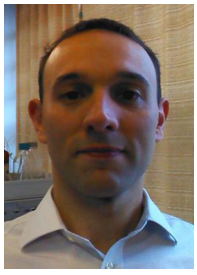

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