ELSEVIER

Journal of Chromatography A, 772 (1997) 91–101

JOURNAL OF CHROMATOGRAPHY A

Determination of the thermodynamic equilibrium constants of water-insoluble (sparingly soluble) compounds by capillary electrophoresis

S. Bellini[a], M. Uhrová[b], Z. Deyl[c,*]

[a]*Glaxo Wellcome S.p.A. Verona, Research and Development Pharmaceutical Dev. Dept., Via Fleming 4, I-37134 Verona, Italy*
[b]*Department of Analytical Chemistry, Institute of Chemical Technology, Technická 5, CZ-16628 Prague 6, Czech Republic*
[c]*Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-14220 Prague 4, Czech Republic*

Abstract

A method based on migration time estimation in capillary electrophoresis for the determination of aqueous pK_a^{th} values of water-insoluble compounds of pharmacological (or environmental) interest is presented. The method requires apparent pK_a estimation at least at two different pH values and at different methanol concentrations in the background electrolyte (usually more points of the methanol concentration vs. pK_a^{th} dependence are determined).

Two automated operational modes were used; in the first, a stepped pH change was applied in the ascending mode (starting with low pH — usually 3.5) reaching, in 0.5 pH units steps, the final value of 5.5, followed by a similar descending pH sequence. This procedure was repeated for different methanol concentrations (w/w). The other operational mode was shortened in the sense that only two buffers were used, typically pH 5.5 and 3.0 (the former being assumed to yield the pH at which the solutes investigated are fully ionized). In all cases, the apparent pH of the methanolic solutions was estimated and the pK_a value was determined by extrapolating the set of data obtained to zero methanol concentration.

Keywords: Ionization constants; Organic acids

1. Introduction

The aqueous ionization constant (pK_a) is a very important physico-chemical property in the pharmaceutical industry [1–4]. Since many compounds of pharmaceutical interest, although possessing a carboxyl group, are only sparingly soluble in water, estimation of pK_a in an aqueous solution may be difficult [5]. Most common methods for pK_a determination include potentiometry or, if the compounds possess a suitable chromophore, then spectroscopic methods can be applied [5]. With water-insoluble compounds, the mixed solvent approach can be exploited. Methanol should be used in the first place because there is a lot of information available about its effect upon pK_a [6–10]. It is believed to be the least error-prone solvent of all possible solvents that can be used for this purpose [1]. The aqueous pK_a value is then estimated by extrapolating the curve constructed from measured apparent pK_a values at different methanol concentrations.

Plots of the pK_a values against the percentage of organic solvent in the solution are rarely completely linear. In most cases, they show a "hockey stick" or "bow" shape. In general, concentrations above 60% (w/w) of the organic solvent are rarely used as beyond this concentration the pK_a vs. percentage of

*Corresponding author.

PII S0021-9673(96)00839-4

the organic modifier dependence frequently attains an S-shaped appearance. Also, at this concentration, ion-pairing starts to interfere with the pK_a estimation and data obtained at such high proportions of the organic solvent in the sample are not suitable for extrapolation to zero e.g. methanol concentration [1,11].

An alternative to pK_a estimation is to use capillary electrophoresis [12]. This approach offers considerable advantages over the two above-mentioned techniques [13,14]. Precise potentiometric titration at low concentration requires a time-consuming preparation of carbonate-free solutions for which automated equipment is only rarely available [15]. Compounds must be available not only in relatively large volumes but also at a concentration higher than 1 mmol/l. Circumventing the solubility problems by working in mixed solvents may induce an error in the extrapolation phase due to different solvation mechanisms and the uncertainty involved in defining the standard state.

Another common approach is to use UV–Vis spectrophotometric titration. The condition to be fulfilled is that the ionized and non-ionized species possess different spectra. Also, these measurements are time-consuming and automated instrumentation is not available [5,15,16].

Of the other possibilities for pK_a measurement, one can think of conductivity-based measurements. These, however, were largely replaced by potentiometry [5]. Isotachophoresis [17–19] and calorimetry [20] can be used in specialized situations. The advantages of using capillary electrophoresis (CE) to determine accurate pK_a values can be summarized as follows:

1. (1) CE requires small amounts of sample at low solute concentrations.
2. (2) No precise information about the sample concentration is needed as long as the response of the UV detector offers a good peak.
3. (3) Calculations are easy and no special demands are put on solvent purity.
4. (4) The only parameter measured is migration time.
5. (5) The equipment available is usually fully automated.

The technique of pK_a determination by means of CE has been worked out by Cleveland et al. [12]. The detection limit for e.g. benzoic acid was 2 μM. Excellent agreement was found between the pK_a values obtained by CE and literature data obtained by other techniques. Although the method of Cleveland et al. [12] documents the possibility of measuring the pK_a values over a large interval, it does not deal with the possibility of estimating pK_a values of water-insoluble (or sparingly soluble) individual compounds. This report extends the existing methodology available for water-soluble compound to solutes that need an organic solvent in order to form a solution.

2. Theory

The thermodynamic equilibrium constant of the dissociation of a weak monoprotic acid is defined as

$$pK_a^{th} = \gamma a_{Z^-} \gamma a_{H^+} \cdot \frac{[H^+][Z^-]}{[HZ]} \quad (1)$$

assuming that the activity coefficient of the undissociated acid, γa_{HZ}, equals one; γa_{Z^-} is the activity coefficient of the anionic species, γa_{H^+} is the activity coefficient of the hydrogen ion. This relation can be rewritten as

$$pK_a^{th} = pH - \log \gamma a_{Z^-} - \log \frac{[Z^-]}{[HZ]} \quad (2)$$

The pH value of the experiment is known, the value of $-\log \gamma a_{Z^-}$ can be calculated on the basis of the Debye-Hückel theory at 25°C according to the equation

$$-\log \gamma = \frac{0.5085 \cdot z^2 \cdot \sqrt{\mu}}{1 + 0.3281 \cdot a \cdot \sqrt{\mu}}$$

$$\text{where } \mu = \frac{1}{2}\sum_{i=1} c_i z_i^2 \quad (3)$$

where a is the hydrated diameter of an ion (in Å) and c is the molarity of the ion, z is the charge of the ion and μ is the ionic strength of the solution. The value of a is unknown and was (in a similar manner to that described in Ref. [12]) assumed to be 5.0 Å. By substituting this equation into Eq. (2), we get

$$pK_a^{th} = pH - \log \frac{[Z^-]}{[HZ]} + \frac{0.5085 \cdot z^2 \cdot \sqrt{\mu}}{1 + 1.6405 \cdot \sqrt{\mu}} \quad (4)$$

In the case of a univalent species, $z=1$, the last part of Eq. (4) attains the form

$$\frac{0.5085 \cdot \sqrt{\mu}}{1 + 1.6405 \cdot \sqrt{\mu}} \quad (5)$$

Our next task is to express the $\log([Z^-]/[HZ])$ part of the equation in terms of electrophoretic mobility calculated from the experimentally determined migration times. Effective mobility of any ionic species m_e is given by the difference of the apparent mobility of the endoosmotic flow

$$m_e = m_{app} - m_{EOF} = \frac{L_c \cdot L_d}{V}\left(\frac{1}{t_{app}} - \frac{1}{t_{EOF}}\right) \quad (6)$$

where L_c is the total length of the capillary, L_d is the length of the capillary to the detector's window, V is the applied voltage and t_{app} and t_{EOF} are migration times of the ionic species under investigation and endoosmotic flow, respectively.

Taking into account that $m_e = \alpha m_a$ (where α is the fraction of the analyte ionized and m_a the electrophoretic mobility of the fully deprotonated species), the ratio of the unprotonated to protonated anionic species can be written as

$$\frac{[Z^-]}{[HZ]} = \frac{\alpha}{1-\alpha} = \frac{m_e}{m_a - m_e} \quad (7)$$

Although the viscosities of methanol and water differ considerably at 289 K (O.89 cP and 0.55 cP), no adverse effects were observed in extrapolating the data.

The final formula for calculations, after substitution, attains the form (valid for monoprotic acids):

$$pK_a^{th} = pH - \log\frac{m_e}{m_a - m_e} + \frac{0.5085 \cdot \sqrt{\mu}}{1 + 1.6405 \cdot \sqrt{\mu}} \quad (8)$$

The values of pK_a^{th} obtained in this way are obviously apparent pK_a values; in order to obtain aqueous pK_a^{th} values, several assays at different methanol concentrations should be performed. In general, the relationship between apparent pK_a^{th} vs. % methanol (w/w) have positive slopes for weak acids and negative slopes for bases.

3. Experimental

3.1. Instrument and analysis arrangement

A Hewlett-Packard HP 3D CE Model G 1600 A (Hewlett-Packard, Cernusco, Italy) electrophoretic apparatus equipped with a UV detector and operated by means of the HP 3D ChemStation programme was used throughout the experiments. Solutes, whose pK_a values were to be estimated, were injected in water–methanol (1:1, v/v); the concentration of the samples was routinely 100 μg/ml and the injection was hydrodynamic (3 s at 50 mbar). The capillary used (Polymicro Technologies, Phoenix, AZ, USA) was 32 cm (26.7 cm to the detector)×50 μm I.D. The effluent was monitored at 220 nm and all runs were performed at 30°C. The capillary was operated at 10 kV with currents not exceeding 50 μA.

In order to equilibrate the columns and minimize the hysteresis effects, the capillary was initially washed with 0.1 *M* NaOH for 1 min followed by a 1-min wash with the run buffer. Each run was allowed to take 40 min, which really was unnecessary as the running times of the solutes were much shorter, however, it offered enough time for the capillary to be stabilized. Two operational modes were used.

In the first, a set of buffers with pH values of 6.5, 5.0, 4.7 and 4.5 was prepared by titrating the 20 mmol/l sodium acetate solution to the required pH value. The samples were run at several methanol concentrations (typically 60, 40 and 20%, w/w). For each methanol concentration, the automated sequence of runs started with the lowest pH value buffer and ascended to the highest value; at this point, a 45-min wash was applied with buffer alone and the descending sequence was started. This approach was applied particularly for compounds with the pK_a value of indomethacin. It also has the advantage of demonstrating the stability (or the extent of hysteresis) of the capillary. Its disadvantage is that the estimation of the pK_a value needs too many electrophoretic runs.

In the second mode, only two buffers were used (pH 5.5 and 3.0) containing various proportions (10–50%, w/w) of methanol, increasing in steps of 5% methanol. The apparent pH after methanol addition was measured and used for further calculations. This arrangement was used for the estimation of the apparent pK_a of benzoic, salicylic, indolecarboxylic and 2-acetylosalicylic acids. It was assumed that in the solutions prepared from pH 5.5 buffer, all of the solutes were completely dissociated, while partial ionization occurred with the set of buffers

prepared by adding the respective amount of methanol to the pH 3.0 buffer. The automated sequence of electrophoretic runs was arranged in such a way that all samples were run at the higher pH first, with a stepped decrease of methanol concentration followed by a set of runs in the low pH buffer, again with a stepped decrease of the organic modifier in the background electrolyte.

A 100-μg/ml benzylalcohol solution mixed with the sample (1:3, v/v) was used as the electroosmotic flow (EOF) marker. No change in the pH of the buffer in the autosampler due to CO_2 absorption was observed before or after analysis; consequently, no additional precautions regarding CO_2 dissolution appeared to be necessary.

3.2. Materials

Methanol, acetic acid, sodium acetate and benzylalcohol were obtained from Carlo Erba (Milan, Italy) and were of p.a. grade; double distilled water was used for preparing both the run buffers and sample solutions.

3.3. Calculations

The apparent pK_a^{th} values were calculated for individual pH values and methanol concentration according to Eq. (4). The migration times at the original buffer (pH 5.5) were considered as migration times (and hence calculated mobilities) of the completely dissociated solute. The value for parameter a in Eq. (3) was taken to be equal 5.0 Å [1].

4. Results

Successful estimation of the aqueous pK_a^{th} for any solute examined depends on the precise estimation of both the EOF and analyte migration times. The overall changes in the migration times with different proportions of methanol are quite dramatic (Fig. 1) and good repeatability of these results is a crucial point. Obviously, a well stabilized capillary is needed to avoid large scatter of the calculated pK_a values. Capillary instability is demonstrated by peak drifting. The absolute differences in migration time are larger with late-occurring peaks and, consequently, are demonstrated more dramatically at higher proportions of methanol in the background electrolyte. This drifting may easily reach ~3 min, as shown in Fig. 2. In most cases, if alkaline washes (typically 0.1 *M* KOH) are used, even for a short time period, the drift of most peaks (provided that the analysis is run in acidic buffers) is positive, i.e. with every consequent run, the migration time increases. However, complete elimination of the alkaline wash usually causes the opposite effect, i.e., a negative drift, as shown in Fig. 3. Also, a well stabilized capillary should exhibit less than 0.06 min migration time difference between subsequent runs (Fig. 4).

In our case, it turned out that avoiding KOH washes in between runs, or even deleting washing completely, is, perhaps, the best remedy to avoid drifting. In this case, a small residual negative drift was usually observed which, however, did not affect the results.

In the first operational mode using ascending pH values of the run buffer followed by the descending pH sequence, the results shown in Fig. 5 were obtained for indomethacin as the model compound (tabulated pK_a value of 4.5). Assuming linear pK_a dependence on the percentage of methanol in the run buffer, the pK_a values for indomethacin that are shown in Table 1 were obtained; as expected, the difference from the tabulated value was the largest for the set of data obtained with 60% methanol in the background electrolyte. The linear relationships are based on averages of the migration time obtained by the ascending and descending operational mode (each particular run was performed in triplicate).

The apparent pK_a values obtained when running the system in the second operational mode, with stepped changes in the concentration of methanol, are summarized in Fig. 6 (data for indolecarboxylic acid, 2-acetoxybenzoic acid, salicylic and benzoic acid are summarized in this figure). The values of aqueous pK_a values obtained by extrapolating the CE measurements to zero methanol concentration are compared with literature data in Table 1. It can be concluded that a good agreement between the aqueous pK_a^{th} values obtained by CE and other methods (mostly potentiometric titration in methanol–water mixtures) was obtained.

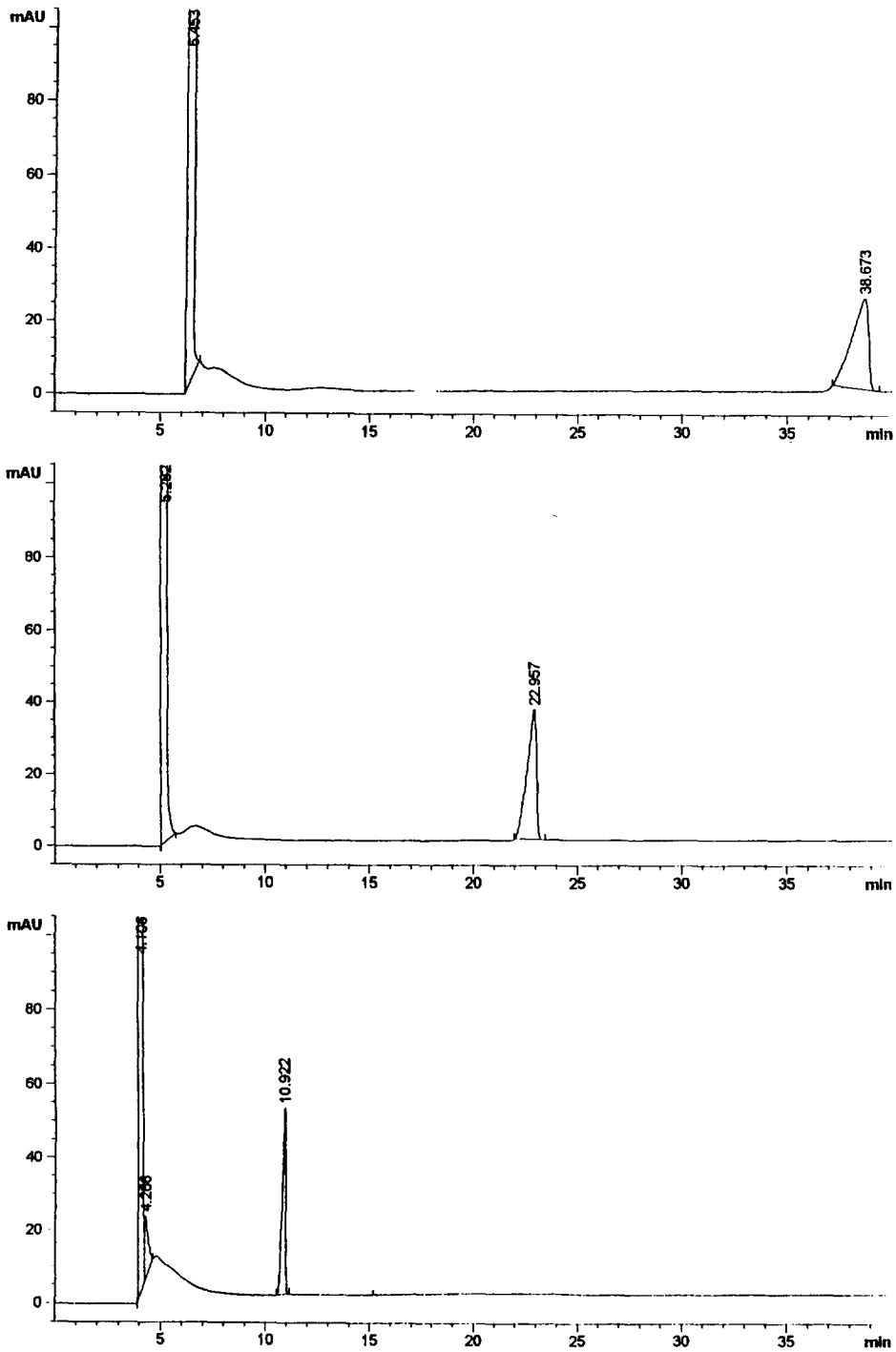

Fig. 1. Changes in benzoic acid migration times with changing methanol concentration in the background electrolyte (50, 40 and 30%, w/w, from the top to the bottom of the figure). The respective amounts of methanol were added to pH 4.5 (20 m*M*) background electrolyte. For experimental details see Section 3. The first peak refers to the EOF marker.

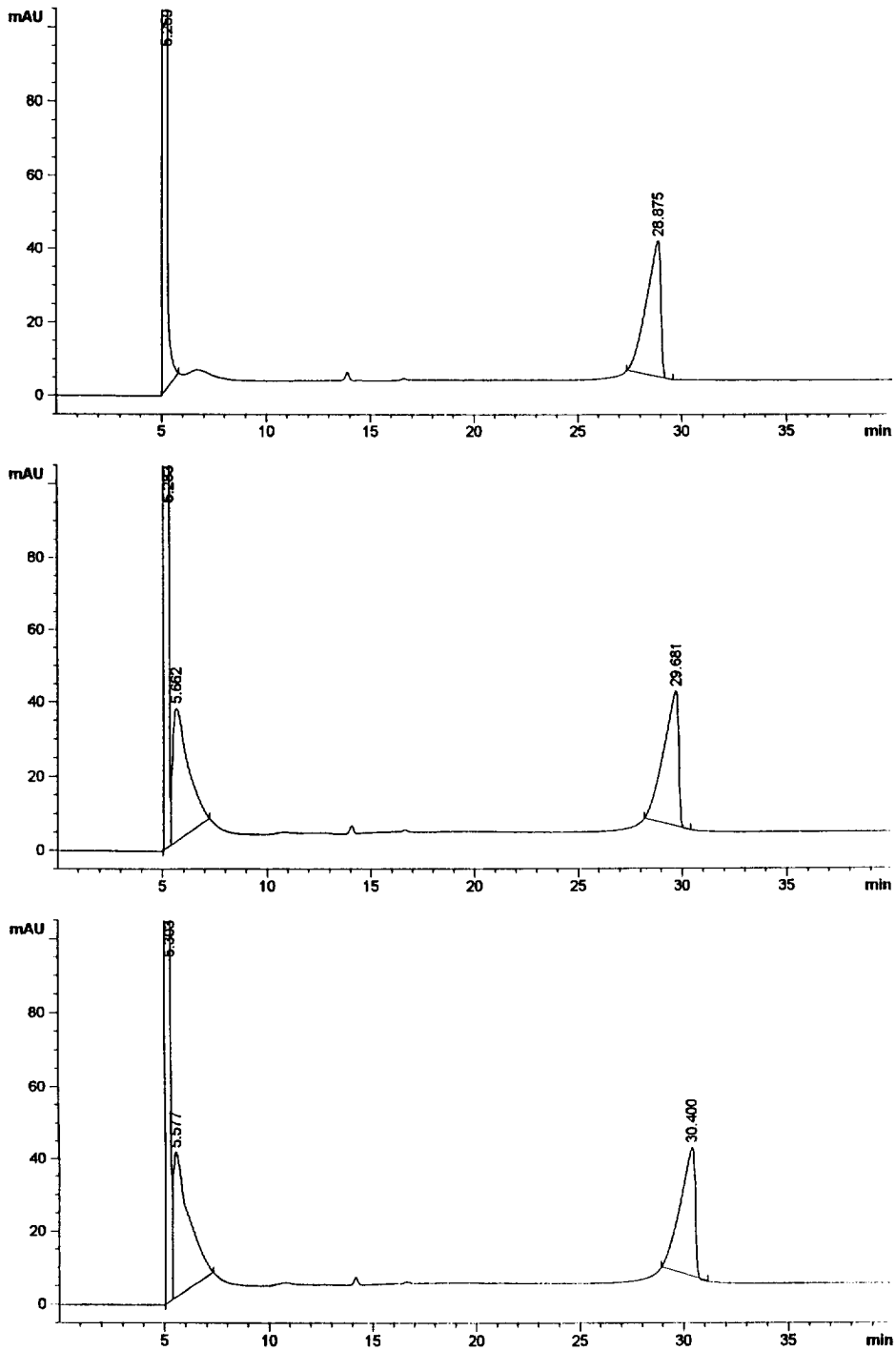

Fig. 2. Drifting of the peak of acetylosalicylic acid in a poorly stabilized capillary: 50% methanol (w/w), 10 kV, capillary, 30 cm (25 cm to the detector). The pH of the background buffer before the addition of methanol was 5.5. Three consequent runs: Top, first run; middle, second run; bottom, third run. The first peak refers to the EOF marker.

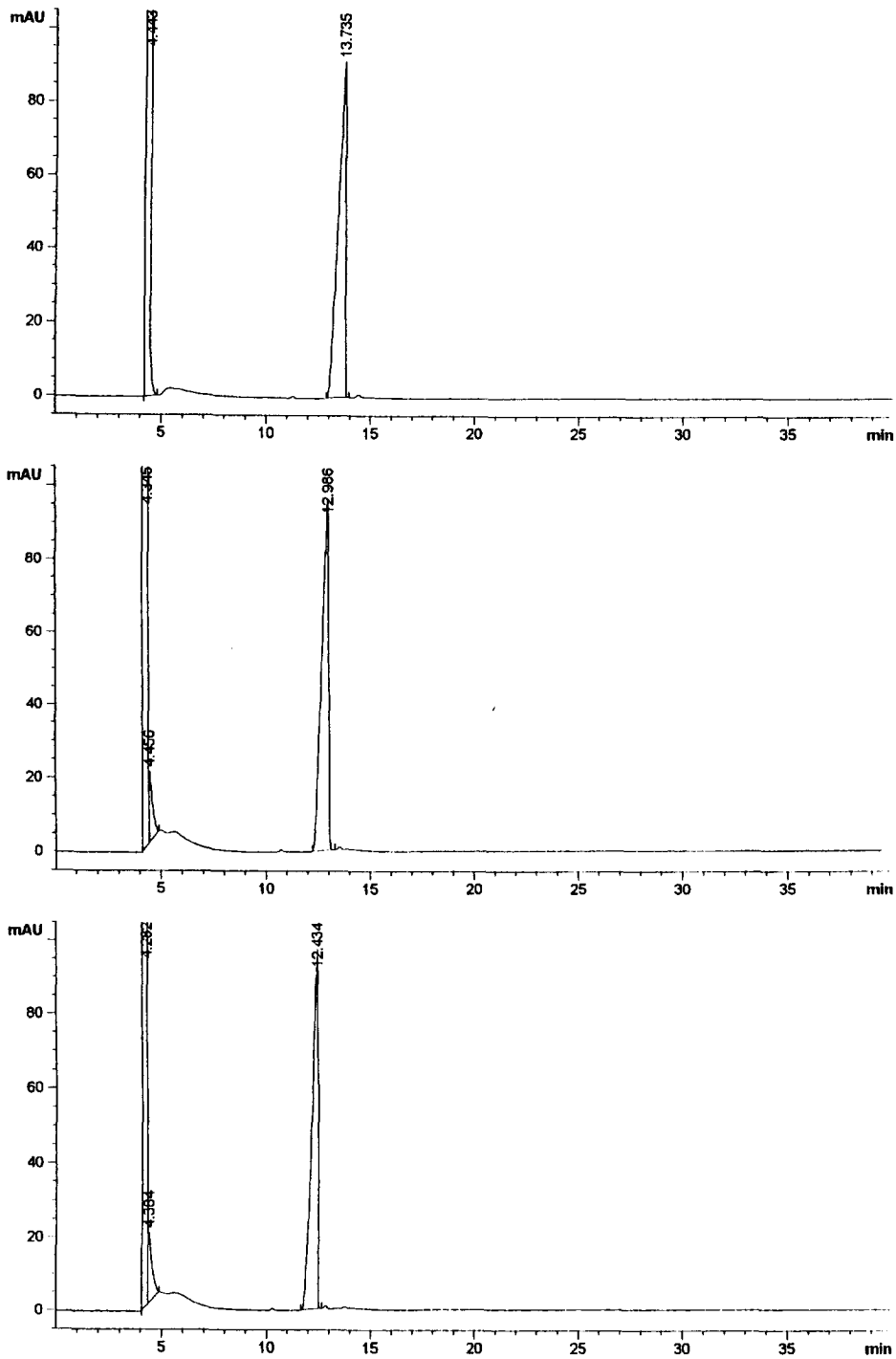

Fig. 3. Reversed drifting of indolecarboxylic acid in 30% (w/w) methanol at the original pH of the background electrolyte, i.e. pH 5.5. No alkali washing was performed between runs. For experimental details, see Section 3. Top, first run; middle, second run; bottom, third run. The first peak refers to the EOF marker.

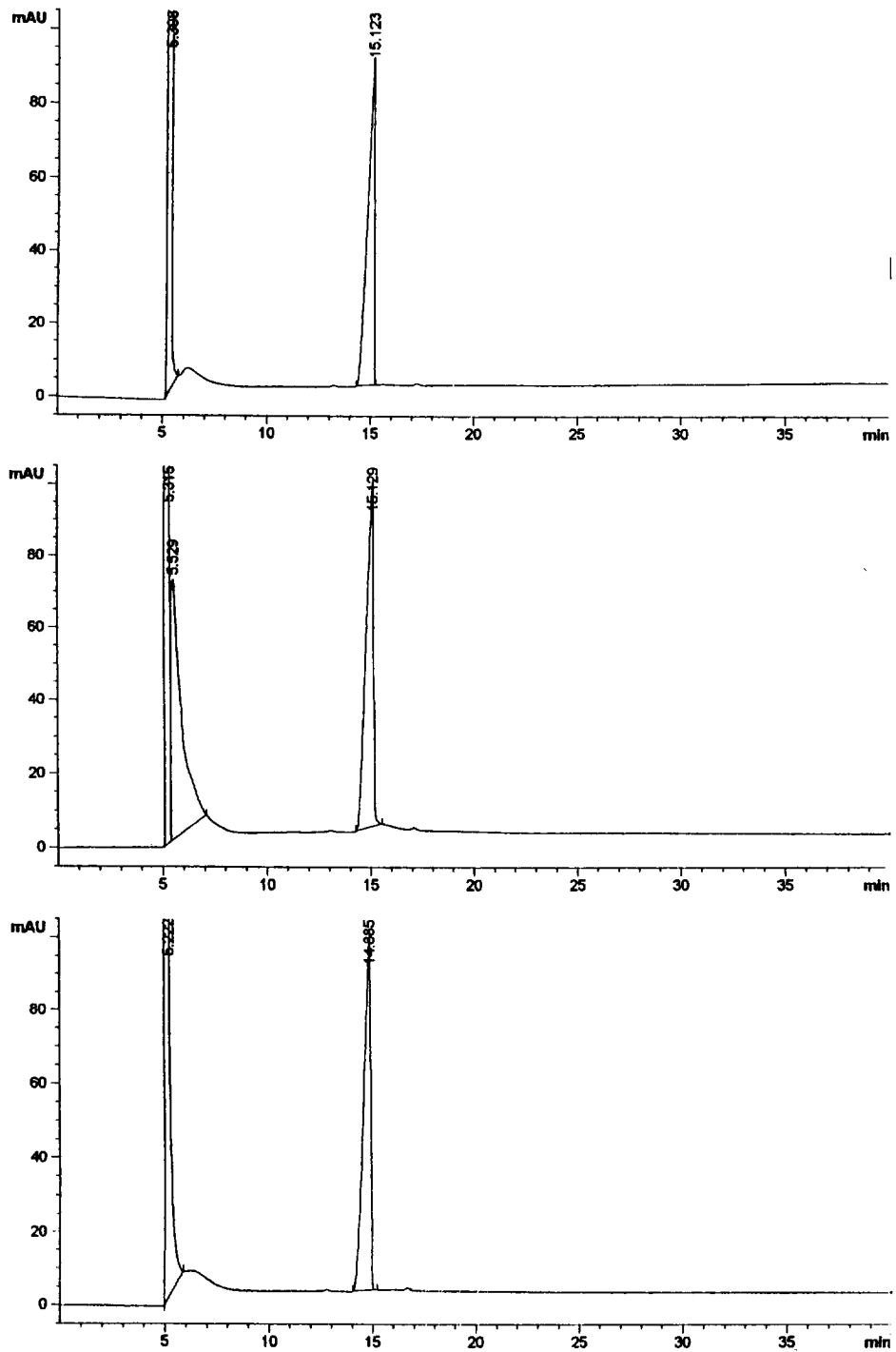

Fig. 4. Well stabilized capillary. Indolecarboxylic acid in buffer, pH 5.5, diluted with 50% (w/w) methanol. Other conditions as described in the text. The capillary was run for about 2 h before starting the experiment without any washing in between runs. The first peak refers to the EOF marker.

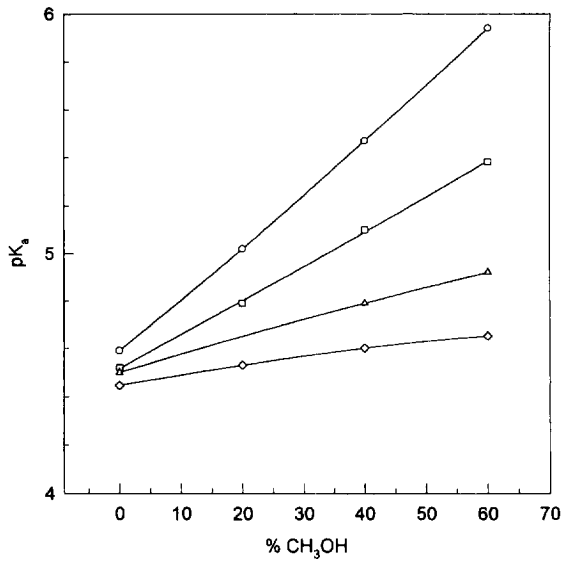

Fig. 5. Extrapolation of the pK_a^{th} values obtained at different methanol concentrations for indomethacin. Individual points were obtained by using a sequence of runs at different pH values of the background electrolyte and represent averages of three consecutive runs, each in the ascending and descending mode. Data obtained with the ascending mode were always lower than those obtained using the descending sequence (SD ±0.03–0.08). They were also higher for higher CH_3OH concentrations in the background electrolyte. Regression times, identification: ○, pH 6.5 (7.871 after methanol addition); □, pH 5.0 (6.301 after methanol addition); △, pH 4.7 (5.658 after methanol addition); ◇, pH 4.5 (5.230 after methanol addition). For extrapolated values (linear regression), see Table 1.

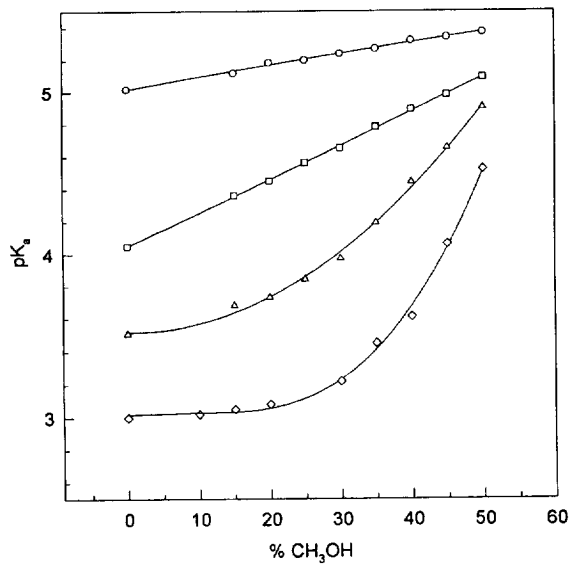

Fig. 6. Extrapolation of pK_a^{th} values obtained at different methanol concentration to zero concentration of the organic modifier. Zero values obtained either by linear regression [for indolecarboxylic (○) acid and benzoic (□) acids] or by polynomial regression fit [for 2-acetyloxybenzoic (△) and salicylic (◇) acids].

5. Discussion

In this communication, we have extended the approach used by Cleveland et al. [12] for pK_a^{th} estimation by capillary zone electrophoresis to the determination of aqueous pK_a^{th} values of water-insoluble (or sparingly soluble) compounds (monovalent protolytes). The approach is that of extrapolating apparent pK_a^{th} values obtained with different methanol concentrations to zero concentration of the organic modifier.

Two experimental arrangements were used. The

Table 1
Aqueous pK_a values for selected carboxylic acids estimated by capillary electrophoresis and compared to the literature data

Acid	Tabulated value	From Yashuda-Sheldovsky plot		After bias correction for acids (according to Ref. [1])	Assayed by CE	
		0–65% CH_3OH extrapolation	30–65% CH_3OH extrapolation		In the absence of CH_3OH (literary data)	In the presence of CH_3OH (this paper)
Benzoic acid	4.19 [21]	3.99 [1]	4.38 [1]	4.04 [1]	4.18 [12]	4.20[a]
Salicylic acid	2.98 [12]	–	–	–	2.96 [12]	3.0[a]
2-(Acetyloxy)benzoic acid	3.49 [21]	–	–	–	–	3.50[a]
Indole-(2)-carboxylic acid	5.04 [22]	–	–	–	–	5.0[a]
Indomethacin	4.50 [22]	–	–	–	–	4.47–4.59[b]

[a]Data obtained by the shortened procedure using stepped concentrations of methanol at two different pH values (3.5 and 5.5 before methanol addition).
[b]Data obtained by using a set of stepped pH values at different methanol concentrations (see Section 3).

first is based on running a particular sample in a series of buffers containing the same proportion of methanol at step-wise increasing or decreasing pH values of the background electrolyte. Such a procedure is gentler to the capillary and can be performed directly with a capillary that has been treated in the usual way, i.e. for 1 min with a 0.1-*M* NaOH rinse followed by a 1-min rinse with the starting buffer at the beginning of the experiment. Both versions, i.e. the descending pH change followed by runs at stepped ascending pH values or vice versa are possible. Of course, because of the well known hysteresis of the fused-silica capillaries, the corresponding values obtained in the descending and ascending mode are slightly different. However, as this approach offers a wealth of data, averaging them appears to eliminate fluctuations due to poor capillary stabilization and its hysteresis. In general, aqueous pK_a values that were very close to the results obtained using potentiometric titration, as reported in the literature, were obtained.

In our hands, it was proven that starting with an ascending pH sequence followed by a descending sequence, i.e. from original background electrolyte at pH values of 3.5 to 5.5 (before methanol addition), followed by a series of runs in the reversed order, is better than starting with the descending sequence followed by a step-wise pH increase.

It has to be stressed that these measurements can be done only using automated equipment, as the error in migration times of hand-operated machines makes the data obtained unsuitable for reliable pK_a^{th} estimation.

From the description of the first procedure, it appears that estimation of the pK_a^{th} value of an unknown compound can be very time-consuming, although quite precise. On the other hand, most of the run time is performed in the fully automated mode which does not require the personal attendance of the operator.

The second approach described here represents a shortened version and is quite satisfactory for practical purposes. It is more rugged and offers sufficiently reliable results in spite of the fact that it is based on a smaller set of data. Theoretically, only two categories of runs are required for the pK_a^{th} determination; at a high and low pH at each methanol concentration (at least four different CH_3OH concentrations are required, giving all in all eight runs performed in triplicate i.e. 24 experiments. Basically, it differs in using only two buffer pH values. This, however, may need several preliminary trials to select the appropriate pH values; for acidic solutes, the question is how acidic must the buffer be for incomplete ionization of the analyte to occur. It has also to be kept in mind that addition of methanol to the background electrolyte may increase the pH value quite considerably. As a matter of fact, the first experimental steps used with the stepped ascending and descending pH change at a given concentration of methanol requires almost no equilibration of the capillary. It appears that the drifts in migration times between the upward and downward pH changes mutually compensate, resulting in more reliable results than when descending methanol concentrations at a given pH value is used.

Although quite a few experiments are still needed, the whole aqueous pK_a^{th} estimation requires only 1 ml of sample containing 100 μg of the solute in most cases.

In most cases, the aqueous pK_a^{th} value lies within the range ± 0.03 pK_a units of the reported values and, in spite of the extrapolation and the need to measure the apparent pH of the metabolic solutions (which is generally felt as a serious source of potential errors), the limits are the same as for water-soluble compounds as reported by Cleveland et al. [12].

Because this procedure is aimed at being used mainly with the compounds considered as prospective drugs, the 100 μg/ml concentration and 1 ml sample size needed for the assay do not represent a limitation and no more methods that were sensitive for detection and/or sample stacking were sought.

Estimating the EOF migration time and the apparent pH of the background electrolyte after methanol addition represents a crucial point in all pK_a^{th} calculations by CE. We have used benzylalcohol (applied in a 1:1 (v/v) water–methanol mixture, the same solvent in which the assayed solutes were dissolved); in spite of this EOF marker being of p.a. quality, we have observed frequently a shoulder on the descending side of the peak. We did not pay attention to this nor to the poor peak shapes that were sometimes obtained for analytes, caused mainly by their poor solubility in solvents containing low proportions of

alcohol. The peak shape at each alcohol concentration could have been optimized, which, in turn, would make the assay of an unknown compound even more time-consuming. For practical purposes, however, the peaks obtained were sufficient and could be used for quite precise aqueous pK_a^{th} estimation.

Cleveland et al. [12] discuss, in their paper, two possibilities for improving the pK_a assays by CE. The first refers to the estimation of the mobility, m_a, of the fully ionized anionic species, the other refers to the estimation of the pH of the buffer in which the run is performed. The first possibility is irrelevant with respect to our data, as this comes into question mainly when buffers of different types (e.g. citrate and acetate) are used during the assay. Admittedly, we neglected the second proposal for improvement as well, mainly because the data we obtained were in concert with the published values of aqueous pK_a^{th} values.

6. Conclusions

The automated pK_a determination procedure at low solute concentrations reported by Cleveland et al. [12] has been extended in this paper to water-insoluble (or sparingly soluble) monovalent protolytes. The basic idea is the same as that applied to the estimation of pK_a^{th} values by means of potentiometric titration (for details see e.g. Ref. [1]): Apparent pK_a^{th} values at different methanol concentrations are estimated based on running the solute to be assayed in background electrolytes containing e.g. 50, 40, 30 and 20% (w/w) concentration of methanol. By plotting the apparent pK_a^{th} values against percentage of methanol (w/w) (recalculated) and by extrapolating the relationship to zero concentration of methanol, the aqueous pK_a^{th} values for a series of compounds were obtained.

It was also shown that, in practice, both operational modes, i.e. a sequence of automated experiments in which individual runs undergo a stepped pH change (at a constant methanol percentage, w/w) or a stepped change of methanol concentration at a given pH (two different values used) offer practically the same results. The advantage of the latter approach is that it is less time-consuming and requires fewer runs. For practical reasons, the latter approach is preferable.

Acknowledgments

The partial support of this work by grant No 203/96/K128 from the Grant Agency of the Czech Republic to two of the co-authors (M.U. and Z.D.) is gratefully acknowledged.

References

[1] A. Afdeef, J.E.A. Comer and S.J. Thomson, Anal. Chem., 65 (1993) 42.
[2] L.Z. Benet and J.E. Goyan, J. Pharm. Sci., 56 (1967) 665.
[3] A. Roda, A. Minutello and A. Firei, J. Lipid Res., 31 (1990) 1433.
[4] W.J. Liman, W.F. Reehl and D.H. Rosenblatt, Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC, 1990.
[5] A. Albert and E.P. Serjeant, The Determination of Ionization Constants, 3rd ed., Chapman and Hall, London, 1984.
[6] A.L. Barcella, E. Grunwald, H.P. Marshall and E.L. Purlee, J. Org. Chem., 20 (1955) 747.
[7] T. Sheldovsky and R.L. Kay, J. Am. Chem. Soc., 60 (1956) 151.
[8] C.L. de Ligny, Recl. Trav. Chim. Pays-Bas, 60 (1960) 731.
[9] C.L. de Ligny, H. Loriaux and A. Ruiter, Recl. Trav. Chim. Pays-Bas, 80 (1961) 725.
[10] L.G. Chatten and L.E. Harris, Anal. Chem., 34 (1962) 1495.
[11] T. Sheldovsky, in B. Pesce (Editor), Electrolytes, Pergamon Press, New York, 1962, pp. 146–151.
[12] J.A. Cleveland, Jr., M.H. Benko, S.J. Gluck and Y.M. Walbroehl, J. Chromatogr. A, 652 (1993) 301.
[13] J.L. Bechers, F.M. Everaerts and M.T. Ackermans, J. Chromatogr., 537 (1991) 407.
[14] J. Coi, J.T. Smith and Z. El Rassi, J. High Resolut. Chromatogr., 15 (1992) 30.
[15] R.F. Cookson, Chem. Rev., 74 (1974) 1.
[16] E.J. King, Acid–Base Equilibria, Pergamon Press, Oxford, 1965.
[17] J. Pospíchal, P. Gebauer and P. Boček, Chem. Rev., 89 (1989) 419.
[18] M. Polášek, B. Gaš, T. Hirokawa and J. Vacík, J. Chromatogr., 598 (1992) 265.
[19] J. Bechers, J. Chromatogr., 320 (1985) 147.
[20] J.J. Christensen, L.D. Hansen and R.M. Izatt, Handbook of Ionization Heats, Wiley-Interscience, New York, 1976.
[21] S. Budavari (Editor), Merck Index, Merck and Co. Inc., Rahway, NJ, 11th ed., 1989.
[22] T. Kariya, J.M. Grisar, N.L. Welch and T.R. Blohm, J. Med. Chem., 15 (1972) 659.