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Ionisation characteristics and elimination rates of some aminoindanones determined by capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) has emerged as an important tool for evaluating the ionisation characteristics of compounds and their corresponding pK_a values. A particular strength of CE in this context is that its relative selectivity allows one to measure the extent of ionisation of materials that are impure. In this study, using CE, we have measured the pK_a values of a series of anti-inflammatory aminoindanones, which underwent degradation to indenone during the course of the determination. We subsequently monitored the elimination reactions measuring remaining indanone over the pH range 2.6–10 at constant ionic strength and temperature. The decomposition of the tertiary amino derivatives was especially fast with first-order half-lives of less than 10 min observed at pH 7.4. The resulting sigmoidal pH rate profiles can be accounted for by assuming unimolecular elimination of the protonated (slow) and neutral (fast) forms of the amines. This study provides further support for the use of CE in evaluating amino ionisation especially in cases where degradation might be expected.

Keywords: Dissociation constants; Aminoindanones; Indanones; Aminoketones; Indenone

1. Introduction

The determination of the ionisation characteristics of candidate molecules is an important step in the drug development process: The tendency of a substance to ionise has important implications for its membrane permeability, receptor interactions and behaviour under analytical conditions. Classically, pK_a values have usually been measured using potentiomeric or spectroscopic methods [1]. In the recent past, capillary electrophoresis (CE) has emerged as an important addition to this armoury [2–23]. Among the most frequently cited merits of CE in this regard are that it requires small amounts of analyte and, since it is also a separative technique, pK_a determination is not affected by impurities or degradants. This paper describes a study where the latter strength was of pivotal importance.

This study was initially directed at the development of analytical methods for a group of highly promising amino indanones (Table 1). These belong to a larger family of anti-inflammatory/anti-allergic compounds developed at our laboratory [24,25]. As part of this work, it was proposed to estimate the ionisation characteristics of test compounds using CE. It was expected that these methods would also prove useful for in vitro metabolism studies and later in pharmacokinetic studies.

During the course of the study it was observed that the test compounds underwent decomposition in aqueous media from about pH 5 upwards, with the evolution of indenone (Fig. 1). The compounds can be viewed as substituted C-Mannich bases and their degradation behaviour was therefore not entirely unexpected [26–30]. However, the rate of some of the elimination reactions seemed particularly rapid, although there is little data in the literature concerning the behaviour of comparable β-amino ketones under aqueous conditions. This report therefore concerns the pK_a determination of a family of indanones by CE and the pH rate profiles for their decomposition. Based on the latter, a mechanism of decomposition is suggested.

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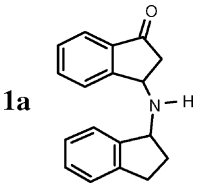
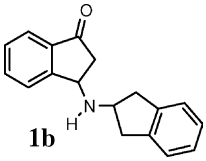
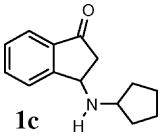
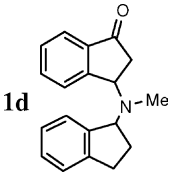
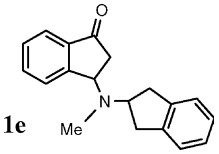
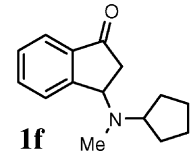
Table 1
pK_a values determined using Eqs. (2) or (11)* and rate data for aminoindanones determined by capillary electrophoresis along with first-order half-lives rate data at pH 3.3 and pH 7.4 ($\mu = 0.154$, 37 °C)

Compound	k_1 (min^{-1})	k_2 (min^{-1})	pK_a*	$t_{1/2}$ (min)		pK_a
				pH 3.3	pH 7.4	
1a 	1.5e−5 ± 0.0002	0.0029 ± 0.0003	6.5 ± 0.3	6931	272	6.4 ± 0.2
1b 	9.3e−5 ± 0.0001	0.0024 ± 0.0001	6.4 ± 0.2	3850	324	6.6 ± 0.1
1c 	4.5e−5 ± 0.0002	0.0024 ± 0.0004	7.4 ± 0.4	36657	495	7.6 ± 0.2
1d 	0 ± 0.003	0.075 ± 0.003	5.8 ± 0.1	3465	9.4	5.5 ± 0.1
1e 	0 ± 0.02	0.10 ± 0.01	5.9 ± 0.4	5728	6.2	6.0 ± 0.2
1f 	6.78e−5 ± 0.01	0.22 ± 0.03	6.3 ± 0.2	2166	3.2	6.5 ± 0.3

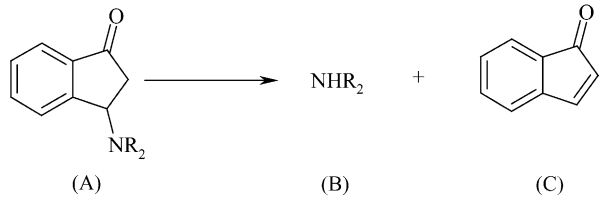


Fig. 1. Scheme showing the decomposition of aminoindanones **1a**–**1f** at 37 °C.

2. Materials and methods

2.1. Materials

All compounds were prepared as detailed in refs. [24,25], and identities confirmed by high-resolution MS [Micromass spectrometer (time-of-flight, electrospray ionisation)] and NMR [20 °C on a Bruker DPX 400 spectrophotometer (400.13 MHz ^{1}H, 100.61 MHz ^{13}C)] both at the Department of Chemistry, Trinity College Dublin. Phosphoric acid (<99%, Fluka), phosphoric acid (85%, BDH), sodium dihydrogenorthophosphate (BDH) and tetrabutylammonium dihydrogenphosphate (Aldrich, 97%) were used for the preparation of running buffers for CE. Citric acid monohydrate (99% ACS, Aldrich), boric acid (M&B) and tripotassium orthophosphate (BDH) were used for the preparation of buffers for kinetic studies and for running buffers used in pK_a determination. Acetonitrile (HPLC grade) from Riedel-de Haën was used for the preparation of stock solutions. Aqueous solutions were prepared with distilled and deionised water (Milli-Q water system, Millipore).

2.2. Determination of pK_a by CE: theory and method

The method of determination of pK_a by CE is based on the relationship between electrophoretic mobility and the degree

of dissociation of a species over a range of electrolyte pHs. When fully ionised, a molecule moves fastest to the electrode of opposite charge, while in its neutral state it moves only under the influence of the electroosmotic flow (EOF). M_e, the effective mobility of an ionic species (at a particular pH), defined as the difference between the apparent mobility and the mobility due to the EOF (M_{EOF}), can be calculated using Eq. (1):

$$M_e = M_{app} - M_{EOF} = \frac{L_c L_d}{V}\left(\frac{1}{t_{app}} - \frac{1}{t_{EOF}}\right) \quad (1)$$

where L_c is the distance from the injection point to the detector (cm), L_d is the total capillary length (cm), V is the applied voltage (V) and t_{app} and t_{EOF} are the migration times (s) of the analyte and a neutral marker compound.

A plot of effective mobility of an ionic species against the pH of the running buffer affords a sigmoidal curve whose inflection point corresponds to the analyte pK_a. The sigmoidal curve has the form:

$$M_e = \frac{M_a - M_0}{1 + 10^{(pH - pK_a^{th})}} + M_0 \quad (2)$$

where M_a represents the absolute mobility and M_0 represents the mobility of the unionised species, which is evidently zero. Non-linear regression of M_e against the pH of the running buffer yields pK_a.

CE was performed using a Beckman P/ACE system 5510 equipped with a UV filter detector set at 200 or 214 nm or a photodiode array detection (DAD)-UV system. Data acquisition was performed using the system Gold and peak areas were recorded at 200 or 214 for the original compound and the parent amine (when applicable). All running buffers were filtered through a 0.45 μm Millipore membrane filter before use.

The electrophoretic mobility was determined using running buffers with an ionic strength of 0.05 and, pH in the range of 2.3–11.5 prepared from two stock solutions: solution A was 0.05 M citric acid monohydrate and 0.02 M boric acid in distilled and deionised water. Solution B was 0.1 M in tripotassium-orthophosphate in distilled and deionised water. Sample solutions were prepared by dilution from stock solutions (acetonitrile, ACN) in pH 3 buffer (for unstable samples) or in water. Dimethyl sulfoxide (DMSO) was added as EOF marker. A 20 cm fused silica capillary was used and maintained at 20 °C. The capillary was rinsed with a HCl (0.01 mM) solution for 10 min prior to the initiation of the tests, followed by water (5 min) and the more acidic running buffer (5 min). Mobilities were then determined in this buffer. Before proceeding to the determination of mobility with the buffer immediately following in the pH scale, the capillary was rinsed with it for a 5-min period. Each sample was tested three consecutive times at each pH and the average mobilities were calculated based on the retention time of the sample and of the EOF. The injection method was by pressure for 3 s and a voltage of 15 kV was applied. Each run lasted for the period necessary for elution of the EOF marker. Batches of not more than six samples were tested with the same running buffer. pH values were measured before and after each test and the average pH taken for the calculations. The two pK_a values corresponding to the ionisation of the NH_3^+ and OH groups of 2-aminophenol (4.78 and 9.97 [1]) were determined on a daily basis as a reference standard.

Each batch was tested three times. The results for mobilities of each sample afforded by the three tests were fitted by non-linear regression to a sigmoidal curve using GraphPad Prism.

2.3. Amine elimination kinetic tests

The CE system had an autosampler with cooling possibility which was modified by connection to a water circulator. Water temperature was set so that a temperature of 37 ± 1 °C was achieved inside the sample vials. Fused silica capillaries were of varying length and an internal diameter of 50 μm. Samples were loaded by pressure injection for 5 s. Runs were carried out at 25 °C and at constant current of 100 or 150 μA in the direction of the cathode. New capillaries were conditioned beforehand with 0.1 M NaOH, followed by deionised water for 5 min and the running buffer for 5 min. Before each run, the capillary was rinsed with running buffer for 1 min, which was a phosphate buffer (pH 3, 100 mM) to which 100 mM of tetrabutylammonium phosphate (TBA) was added.

The disappearance of test compounds was studied in the pH range 2.6–10. The working buffers were prepared from the stock solutions described in Section 2.2. The two solutions were mixed and diluted in the necessary proportions to achieve the desired pH and ionic strength. For ionic strengths above 0.6, NaCl was used. Typically, stock solutions of the compounds under investigation of approximately 1–5 mg/ml in acetonitrile were prepared. Aliquots of stock solutions (20–100 μl) were diluted in 1–3 ml of working buffer warmed at 37 °C to obtain solutions with a final concentration of approximately 100 ppm. Each solution was introduced into the autosampler of the CE warmed at 37 °C and injections started immediately. Decay in concentration of test compounds and/or increase in the concentration of free amines was followed by the decrease/increase in the corresponding peak areas as detected at appropriate wavelength at time intervals as exemplified in Fig. 2 for compound **1b**.

3. Results and discussion

3.1. Determination of pK_a values

The experimentally determined pK_a values for the amines appear in Table 1. During the course of the determinations significant degradation of some of the test compounds was observed, with the formation of a new amino compound along with an apparently neutral compound. The selection

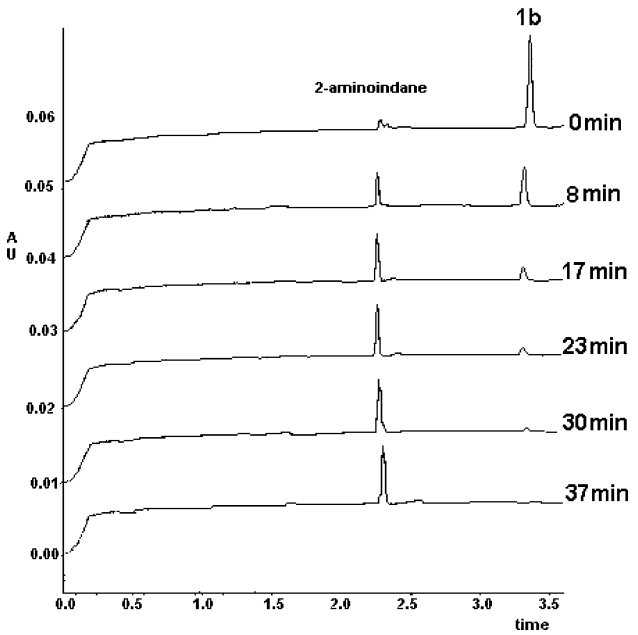


Fig. 2. Elimination of 2-aminoindane from compound **1b** at pH 7.3.

of CE in the present case was therefore vindicated, because the migration of the parent amine, which is a function of its ionisation characteristics, could be easily followed even in the presence of the evolving degradants. The pK_a values fell in the range 6–7.5. As expected, the secondary amines **1a–c** were more basic than the tertiary amino compounds **1d–f**. In both classes the less bulky cyclopentylamine compound exhibited the highest pK_a.

3.2. Elimination kinetics

When the test compounds were observed to degrade it was speculated that this might be due to the elimination of the amine moiety with formation of indenone (Fig. 1) because a common neutral compound was observed to increase in concentration over time in aqueous solutions of the parent amines. The identity of the eliminated amines was confirmed by injection of external standards as appropriate. The identity of the indenone side product was confirmed by its DAD-UV spectrum and migration time (CE), which coincided with that of an authentic sample of indenone prepared by treating bromoindanone with triethylamine in diethyl ether.

The equilibrium between β-aminoketones, α,β-unsaturated ketones and the parent amines in alcohol solution is well known and has been studied in the past [30]. There is, however, very little evidence of studies on the same kind of chemistry in aqueous solutions and this may be related to the relative instability of the compounds in solution (organic or aqueous) and in solid state [30]. In order to obtain an insight into the mechanism of the degradation it was decided to determine the relationship between elimination rate and buffer pH.

The disappearance of test compounds at 37 °C in aqueous solution over the pH range 2.6–10 was monitored using CE. The degradation of compounds **1a**, **1b** as well as the tertiary amine **1e** was investigated at five different ionic strengths in the range $\mu = 0.5$–1.0 (pH 7.4, 37 °C). The degradation of the secondary amino compounds did not exhibit a significant dependence on ionic strength but a small effect was observed in the case of **1e** ($k_{obs} = 0.9\mu + 0.01$). In determining the overall relationship between aqueous buffer pH elimination rate, the ionic strength was maintained at a constant value ($\mu = 0.154$).

In general, the disappearance of compounds followed apparent first-order kinetics over several half-lives. First-order plots were constructed from the natural logarithm of remaining compound versus time and the half-lives estimated using Eq. (3):

$$\ln[A] = -k_1 t + \ln a_0 \tag{3}$$

This approach did not satisfactorily model the behaviour of compound **1d** at pH values above 5 because the elimination reaction did not proceed to completion. This was considered to be due to the occurrence of the reverse second-order reaction involving the addition of the eliminated amine to the indenone. In such cases, the first-order rate constant (k_1) of the forward reaction may be estimated based on the initial and equilibrium concentrations of the species involved by Eq. (4) [31]:

$$\ln\left(\frac{a_0 b_{eq} + b a_{eq}}{a_0(b_{eq} - b)}\right) = \frac{2a_0 - b_{eq}}{b_{eq}} k_1 t \tag{4}$$

Since the concentration of B at equilibrium (b_{eq}) and at time t (b) can be related to the original concentration of A by $b_{eq} = a_0 - a_{eq}$ and $b = a_0 - a$, Eq. (4) can be rewritten strictly as a function of the concentration of A

$$\ln\left(\frac{{a_0}^2 - a a_{eq}}{a_0(a - a_{eq})}\right) = \frac{a_0 + a_{eq}}{a_0 - a_{eq}} k_1 t \tag{5}$$

The graphical representation of this equation should provide a straight line for times preceeding equilibrium. The first-order rate constant can be derived from the slope from:

$$k_1 = \text{slope}\frac{a_0 - a_{eq}}{a_0 + a_{eq}} \tag{6}$$

Rate data for all compounds at high and low pH values at 37 °C are presented in Table 1. The pH–rate profiles for selected compounds are presented in Fig. 3. All compounds were vulnerable to decomposition at high pH but stable in aqueous solution at low pH. The difference in behaviour at low and high pH was most marked for the tertiary amino indanone derivatives of the secondary amines. Whereas the rates of decomposition for the two classes was broadly similar at low pH values, at pH 7.4 the rate of amino release from the tertiary amino compounds was about an order of magnitude higher than from the secondary amines (Table 1). Tetrabutylammonium phosphate was used to obtain peaks with a

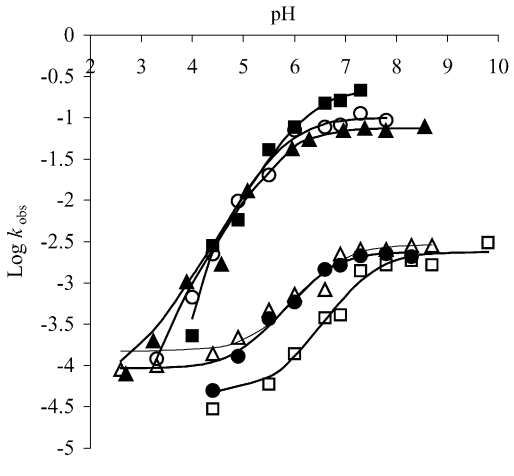


Fig. 3. Plot showing the pH–rate profiles for the disappearance of compounds **1a** (△), **1b** (●) and **1c** (□), along with the related tertiary amines **1d** (▲), **1e** (○), and **1f** (■) at 37 °C and $\mu = 0.154$.

symmetrical shape during the kinetic studies. Because it also suppresses the EOF, the detection of the neutral indenone was not possible but its presence as a decomposition product was confirmed by running some test samples without TBA.

The pH–rate profiles for the β-aminoindanones have a sigmoid shape. This can be accounted for by assuming unimolecular decomposition of the protonated and unionised forms of the compound:

$$R_3NH^+ \xrightarrow{k_1} R_2NH_2{}^+ \tag{7}$$

$$R_3N \xrightarrow{k_2} R_2NH \tag{8}$$

No specific acid catalysis was detected, as this would be revealed by an increase in the rates of degradation at low pH. Base catalysis may have some contribution at higher pH but this is not evident in the pH range of the pK_a values for individual amines. The overall observed rate constant is dependent solely on the individual degradation rates of the ionised and unionised base and on the degree of ionisation at each particular pH:

$$k_{obs} = k_1 \frac{a_{H^+}}{a_{H^+} + K_a} + k_2 \frac{K_a}{a_{H^+} + K_a} \tag{9}$$

where k_1 and k_2 are the first-order rate constants for spontaneous degradation of the protonated and unionised compound, respectively. The terms $a_{H^+}/(a_{H^+} + K_a)$ and $K_a/(a_{H^+} + K_a)$ represent, respectively, the protonated and free base fractions of the compound at each pH.

This expression can be rearranged to:

$$\log\left(\frac{k_{obs} - k_1}{k_2 - k_{obs}}\right) = \log K_a - \log a_{H^+} \tag{10}$$

and can be reorganised in the form of a particular case of a Boltzmann sigmoid:

$$k_{obs} = k_2 - \frac{k_2 - k_1}{10^{(pH - pK_a)} + 1} \tag{11}$$

Eq. (11) was fitted to the experimental points of the pH-rate coefficient profiles of each compound by non-linear regression. Fitting was performed using GraphPad Prism for all compounds, generally in the range comprised between the lower degradation rate observed at low pH, and the highest at pH under 8. The k_1 and k_2 values obtained in this manner are presented in Table 1, along with the pK_a values, determined from the inflection point. These are in good agreement with pK_a values determined using the electrophoretic mobility and Eq. (1) (Table 1); because data for the latter determination are obtained more directly, the corresponding pK_a values are likely to be more accurate. In the pH–rate profiles for compounds **1a**–**1f** presented in Fig. 3, the line was generated using Eq. (11) along with the pK_a, k_1 and k_2 values appearing in Table 1.

4. Conclusion

The present study provides further support for the use of CE in the determination of the ionisation constants of amino compounds. The CE approach was particularly advantageous in the present instance because of the unexpected and rapid decomposition of some of the test compounds. The elimination of the indanones obeyed unimolecular kinetics in the pH range studied. In one instance, there was evidence of an equilibrium being established at higher pH values. The rapid elimination of the tertiary amines along with their high stability at low pH has prompted us to examine amino indanone formation as a means of transiently masking secondary amines. This might have application in protecting group chemistry or in prodrug design.

Acknowledgements

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