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Rapid spectrophotometric pH measurements based on the dual-wavelength technique

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Abstract: A spectrophotometric method for the measurement of pH in solutions of lidocaine hydrochloride (Xylocaine®) for injection is presented. 4-Nitrophenol is used as an indicator for determinations of pH in the range 6.5–7.0. The method was found to be faster than the conventional potentiometric method, mainly due to the utilization of a computer-controlled photodiode array spectrophotometer for the measurements. A further advantage with the spectrophotometric method is that errors arising from varying liquid junction potentials are avoided.

Keywords: *pH determination; spectrophotometry; indicators; pharmaceutical analysis; photodiode array spectrophotometer; lidocaine hydrochloride; 4-nitrophenol; liquid-junction errors.*

Introduction

The potentiometric measurement of pH is a well established technique in the analytical laboratory. A comprehensive review of the pH concept and the theory of pH measurements was presented recently by Bates [1].

Apart from the alkaline error of the glass electrode the most likely source of errors in pH determinations seems to be the liquid junction of the reference electrode [1–3]. The residual liquid-junction potential can be neglected usually when the ionic strength of the sample is less than 0.1 [1], but clogging or adsorption phenomena in the ceramic plug can cause large liquid-junction potentials [2, 3]. Unreliable pH values will, of course, be obtained also when sample constituents, e.g. silica [4], are adsorbed on the surface of the glass electrode.

Spectrophotometric methods are often preferred for the measurement of pH or pH changes [5–7] in living cells or in connection with enzymatic analysis [8]. These are examples of situations where the response of the pH electrode is too slow. Spectrophotometric determinations of pH might also be considered when malfunction of the reference electrode is suspected.

During pH measurements in different solutions of lidocaine hydrochloride (Xylocaine®) for injection in the authors' laboratory, it was observed that the response time of

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the electrodes increased during their lifetime. A lag time of 5–10 min before a stable pH value could be obtained was not uncommon for older electrodes. Accordingly, frequent calibration of the electrodes was necessary, so that the pH determination of a series of samples could be rather time-consuming. A spectrophotometric method for the determination of pH in Xylocaine® injections was therefore developed in order to overcome the drawbacks of the potentiometric method.

Since a photodiode array spectrophotometer interfaced to a personal computer was available in the laboratory, it was proposed that a lot of time could be gained if this instrument were used both for measurement and calculation. An absorbance spectrum can be measured in less than 1 s with a multichannel spectrophotometer and on-line data acquisition and processing allows almost instantaneous presentation of the analytical result. A detailed review of the merits of multichannel detection in pharmaceutical analysis was given recently by Fell *et al.* [9].

Theoretical aspects

A list of the symbols used is given in Table 1.

The spectrophotometric measurement of pH is based on the well-known equation [10]:

$$\mathrm{pH} = \mathrm{p}K'_{\mathrm{HIn}} + \log([\mathrm{In}^-]/[\mathrm{HIn}]). \tag{1}$$

Since HIn and In^- have different absorption spectra and the ratio $[In^-]/[HIn]$ changes with pH, the absorption spectrum of an indicator will also change with pH. A typical example is shown in Fig. 1.

If the absorbance is measured at two different wavelengths the following equations will apply:

$$A_{(1)} = \varepsilon_{\mathrm{HIn}(1)} \times l \times [\mathrm{HIn}] + \varepsilon_{\mathrm{In}^-(1)} \times l \times [\mathrm{In}^-] \tag{2}$$

$$A_{(2)} = \varepsilon_{\mathrm{HIn}(2)} \times l \times [\mathrm{HIn}] + \varepsilon_{\mathrm{In}^-(2)} \times l \times [\mathrm{In}^-]. \tag{3}$$

Division of equation (2) by equation (3) gives, after rearrangement:

$$\frac{[\mathrm{In}^-]}{[\mathrm{HIn}]} = \left\{\frac{A_{(2)} \times \varepsilon_{\mathrm{HIn}(1)} - A_{(1)} \times \varepsilon_{\mathrm{HIn}(2)}}{A_{(1)} \times \varepsilon_{\mathrm{In}^-(2)} - A_{(2)} \times \varepsilon_{\mathrm{In}^-(1)}}\right\} \tag{4}$$

Table 1
Symbols used

a_{H^+}	= hydrogen ion activity
B and HB^+	= amine in non-protonated and protonated form respectively
HIn and In^-	= indicator in acidic and basic form
[HIn]	= molar concentration of HIn
$C^\circ_{HB^+} = [HB^+] + [B]$	= total molar concentration of the amine
$K'_{HIn} = a_{H^+} \times [In^-] \times [HIn]^{-1}$	= acidity constant of HIn
$K_{HBIn} = [HBIn] \times ([HB^+] \times [In^-])^{-1}$	= ion-pair association constant of HBIn
$A_{(1)}$ and $A_{(2)}$	= measured absorbance at wavelength 1 and 2 corrected for background absorption
λ_{max}	= wavelength of maximum absorption
$\varepsilon_{HI(1)}$	= molar absorptivity of HI at wavelength 1
l	= solution path length
μ	= ionic strength

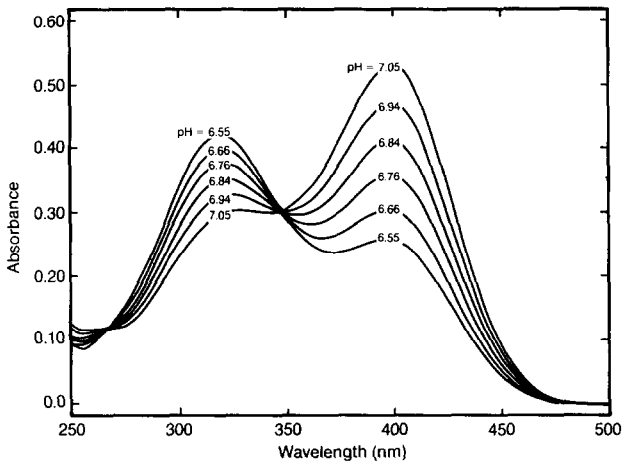

Figure 1
Absorption spectrum of 4-nitrophenol at different pH values. $C^{\circ}_{HIn} = 5.5 \times 10^{-5}$ M (μ = 0.15, 25°C).

which can be combined with equation (1). This equation will then assume the following form:

$$pH = pK'_{HIn} + \log \left\{ \frac{A_{(2)} \times \varepsilon_{HIn(1)} - A_{(1)} \times \varepsilon_{HIn(2)}}{A_{(1)} \times \varepsilon_{In^-(2)} - A_{(2)} \times \varepsilon_{In^-(1)}} \right\} . \quad (5)$$

Accordingly, if $A_{(1)}$ and $A_{(2)}$ are measured and pK'_{HIn} and the ε-values are known, pH can be calculated with equation (5). A similar approach was used by MacDonald *et al.* [6] for the measurement of pH changes in living cells.

A comparison of equation (5) with equation (6), which is valid for measurements at a single wavelength shows that when using the dual-wavelength approach, it is not necessary to know the concentration of the indicator, C°_{HIn}.

$$pH = pK'_{HIn} + \log \frac{A_{(1)} - \varepsilon_{HIn(1)} \times l \times C^{\circ}_{HIn}}{\varepsilon_{In^-(1)} \times l \times C^{\circ}_{HIn} - A_{(1)}} . \quad (6)$$

This is advantageous from a practical point of view, since sample and indicator solution volumes do not have to be accurately measured before mixing. Besides, indicators with a varying water content (e.g. dinitrophenols) can be used directly in the preparation of the solutions since an assay of the content is not needed.

Experimental

Apparatus

The spectrophotometric measurements were carried out with a photodiode array spectrophotometer (HP 8450 UV/VIS Spectrophotometer, Hewlett–Packard Co.) which was controlled by a personal computer (HP 85, Hewlett–Packard Co.). A program was written in BASIC for the data acquisition and processing. The number of samples,

sample identification and ambient temperature were input to the program, which was also provided with the necessary constants and algorithms for the calculations. Quartz cuvettes (1-cm) were used throughout this work. The potentiometric measurements were made with a pH meter (PHM 26, Radiometer A/S) using glass and saturated calomel electrodes (G202B and K401, respectively, Radiometer A/S). A silver/silver chloride reference electrode without liquid junction (P501, Radiometer A/S) was also used in some measurements. The titrant was added with a 1-ml motor-driven piston burette (Dosimat E535, Metrohm AG).

Chemicals and reagents

Lidocaine hydrochloride was of pharmacopoeial grade. Bromothymol blue (BTB), 4-nitrophenol (PNP) and tris(hydroxymethyl)aminomethane (Tris), all of *pro Analysi* grade, were obtained from Merck AG and used as received. An assay of PNP by potentiometric acid–base titration gave a content of 99.8% w/w. Water free of carbon dioxide was used in the preparation of all solutions. The titrants, 1 M sodium hydroxide and 1 M hydrochloric acid, were prepared from Titrisol® ampoules (Merck AG). Solutions of lidocaine–HCl and Tris–HCl of known pH were prepared by adding a calculated volume of 1 M sodium hydroxide to solutions of the amine hydrochlorides. All solutions were adjusted to an ionic strength of 0.15 with sodium chloride in order to match the ionic strength of the sample solutions (isotonic saline).

Determination of molar absorptivities and acidity constants

The measurements were made on solutions thermostatted to 20, 25 or 30°C. The ε-values of PNP were determined in 0.15 M HCl, 0.15 M NaOH or in 0.15 M NaCl (isosbestic ε-value) using indicator concentrations from 3×10^{-5} to 8×10^{-5} M as given in Table 2. The pK'_{HIn} and pK'_{HB^+} values were determined according to Albert and Serjeant [11] and are presented in Table 3. The pK'_{HIn} value of PNP was determined spectrophotometrically using phosphate buffer ($\mu = 0.15$) with pH = 6.5–7.1 and an indicator concentration of 5.5×10^{-5} M. The pK'_{HB^+} values of lidocaine–HCl and Tris–HCl were determined by potentiometric titration with 1 M NaOH or 1 M HCl. The pK'_{HB^+} values given in Table 3 are the mean values of three titrations using different total concentrations of the amines (2×10^{-3}, 5×10^{-3} and 0.01 M, respectively).

Procedure for the spectrophotometric determination of pH

Add 2.5–3.0 ml sample solution into the sample and reference cuvettes. Balance the cuvettes by measuring the absorbance in the range 250–500 nm. Add one mini-drop (0.025 ml) of 0.1% PNP solution into the sample cuvette and mix. Measure the absorbance at 317 (or 347) and 400 nm. Calculate the pH according to equation (5), using the pK'_{HIn} value that is valid at the temperature of measurement (cf. Table 3). In the present work the interpolation between the pK'_{HIn} values was carried out by an algorithm included in the interactive BASIC program which controlled the diode array spectrophotometer.

Results and Discussion

Choice of indicator and wavelength for measurements

The pH of lidocaine HCl (Xylocaine®) injections should be within the range 5.0–7.0 [17]. The indicators BTB and PNP, with transformation pH ranges of 6.2–7.6 and

Table 2
Molar absorptivities of 4-nitrophenol (HIn) and 4-nitrophenolate (In^-)

Wavelength (nm)		$\varepsilon(10^3\ M^{-1}\ cm^{-1})$		
λ_{max}	Isosbestic wavelength	HIn	In^-	Reference
317	—	9.72	1.48	This work
317	—	9.72	1.39	[12]
—	347	5.51	5.51	This work
—	350	5.5*	5.5*	[12]
400	—	0.0839	18.3	This work
407	—	—	18.33	[12]
404	—	—	17.9	[13]

* Estimated from Fig. 1 in [12].

Table 3
Acidity constants of lidocaine, PNP and Tris

Acid	pK'_{HB^+} or pK'_{HIn} 20°C	25°C	30°C	Reference
Lidocaine–HCl	—	7.99	—	This work
	—	7.99	—	[14] $\mu = 0.1$
4-Nitrophenol	7.06	7.02	6.97	This work
	7.22	7.15, 7.16*	7.10	[13, 15], pK_a values
Tris–HCl	—	8.18	—	This work
	—	8.21	—	[16], $\mu = 0.1$

* A pK'_{HIn} value of 7.04 is obtained by use of Davies' equation, cf. [13].
Ionic strength: 0.15.

5.0–7.0 [10], were therefore investigated. BTB could not be used in these samples, however, since a precipitate — probably an insoluble amine/indicator complex, cf. [18] — was formed.

The molar absorptivities at the wavelengths where the acid and base forms of PNP have maximum absorbance are given in Table 2 together with the isosbestic ε-value. The temperature dependence on the ε-values was found to be negligible within the range 20–30°C.

The acidity constants of PNP, lidocaine–HCl and Tris–HCl are given in Table 3. The decrease of the pK'_{HIn} value of PNP with increasing temperature should be considered when a high accuracy in the pH determinations is required.

Unfortunately, the background absorbance of the Xylocaine® sample solutions was too high ($A > 3$) to allow reliable measurements of the absorbance at 317 nm, i.e. λ_{max} for HIn. At wavelengths higher than 340 nm, however, the sample background absorbance was less than 0.02 absorbance units. The isosbestic wavelength 347 nm and λ_{max} for In^-, 400 nm, were therefore chosen for the measurements.

Sources of error and interferences

The accuracy of the method will depend largely on the values of ε and pK'_{HIn}, which in this case serve as secondary 'pH standards'. A systematic error of 0.03–0.05 log units is

probably included in the pK'_{HIn} values in the present work, since the ionic strength of the sample solution was higher than that of the buffer solutions, cf. [19]. This residual liquid-junction effect is, however, also included in all the potentiometric pH measurements in the present work. The results obtained with both methods are therefore comparable since the error in the standardization is the same in both cases.

The addition of an indicator to the samples may cause pH changes if the buffer capacity of the sample solutions is too low. The samples examined in the present work were only slightly buffered, the buffer capacity being 0.0016–0.012 M/pH (calculated according to [20] by use of $C^o_{HB^+}$ = 0.0185–0.074 M, pH = 6.6–6.9, pK'_{HB^+} = 7.99). Thus even relatively low concentrations of PNP might be expected to affect the pH of the sample solutions. Measurements on the sample solutions showed that the pH change was $\leq$0.01 pH provided that C^o_{HIn} did not exceed 3×10^{-4} M, which is in agreement with the values expected from the buffer capacity. Furthermore, a 10% dilution of the sample solutions gave no measureable change of the pH. The addition of 0.025 ml 0.1% PNP to a sample volume of 2.5–3.0 ml gives a dilution of only about 1% and $C^o_{HIn} \sim 0.6 \times 10^{-4}$ M, which is well within the allowable limits.

Erroneous pH values will be obtained also if the PNP anion forms ion-pairs with cations present in the sample solutions. The reaction $HB^+ + In^- = HBIn$, for example, will increase the total concentration of the anionic form of PNP; hence too high a pH value will be recorded, cf. equation (1).

Since association between indicators such as BTB and methyl orange and quaternary ammonium ions or alkali metal ions have been reported [18, 21] it was necessary to check whether this side reaction occurred in the validation of the spectrophotometric method.

Figures 2a and 2b show some of the results obtained from measurements on Tris–HCl and lidocaine–HCl solutions of known pH. In addition to the measurements shown in Fig. 2b, spectrophotometric pH determinations were also carried out at pH = 6.90, the results being 0.03–0.05 pH higher than the calculated values. The good agreement between the calculated and the spectrophotometrically-determined pH values of Tris–HCl in Fig. 2a indicate that the formation of NaIn and HBIn is negligible in this case. If ion-pair formation took place the measured pH would increase with the concentration of HB^+ or Na^+, unless a conditional acidity constant, K^*_{HIn} [14], such as:

$$K^*_{HIn} = K'_{HIn} \times (1 + K_{HBIn} \times [HB^+]) \quad (7)$$

were used instead of K'_{HIn} in equation (1).

In the lidocaine solutions (Fig. 2b) the spectrophotometric pH values were 0.02–0.05 pH units higher than the calculated values. The deviations obtained may be an indication of ion association between HB^+ and In^-. An increase of $[HB^+]$ from 0.05 to 0.15 M would decrease pK^*_{HIn} by 0.02–0.06 log units if $K_{HBIn} = 1$, cf. equation (7), which is consistent with the observed pH changes in the lidocaine solutions. From a practical point of view, however, a difference in results of 0.05 pH should be acceptable. Besides, in cases where the formation of HBIn or NaIn is significant, it should be possible to compensate for this side reaction by the use of equations (7) and (5), provided that K_{HBIn} and $[HB^+]$ are known.

Figures 2a and 2b also show that the difference between the calculated and the potentiometrically determined pH values increases with the concentration of the amine hydrochlorides. This is probably due to a changing liquid-junction potential since this effect was much less pronounced when a reference electrode without liquid junction was used (Fig. 2b).

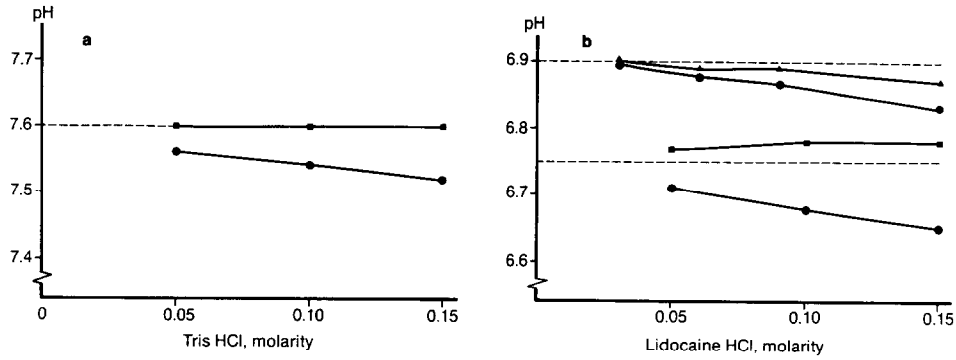

Figure 2(a) and (b)
Comparison of spectrophotometric and potentiometric pH determinations. Dotted lines: calculated pH values (μ = 0.15, 25°C). ■ Spectrophotometry, equation (5). ● Potentiometry, reference electrode with liquid junction. ▲ Potentiometry, reference electrode without liquid junction.

Applications

The spectrophotometric method for the determination of pH was tested on Xylocaine® injections with different contents of lidocaine–HCl (Table 4). The potentiometric pH values were 0.04–0.10 pH units lower than those determined spectrophotometrically. A paired comparisons test [22] indicated that there was a systematic difference between the two methods (n = 14, p = 0.975, $t_{calculated}$ = 13.2, $t_{critical}$ = 2.16). The difference was more pronounced at higher concentrations of lidocaine–HCl and is probably due to a changing liquid-junction potential.

An estimate of the precision of the methods was obtained from pH measurements of one sample, performed by the same operator, but on different days. The standard deviation (n = 10) was $\leq$0.03 pH for the potentiometric, and $\leq$0.02 pH for the spectrophotometric method.

According to an F-test [22] there was no significant difference with regard to the precision of the methods: (n = 10, p = 0.975, $F_{calculated}$ = 1.66, $F_{critical}$ = 4.03).

The spectrophotometric method might also be useful for pH determinations in other samples, where pH electrodes show slow response or give erratic values. Further possibilities with the proposed technique are to use indicator mixtures in order to measure pH over a wider range and to utilize the multi-component analysis program of the diode array spectrophotometer for the calculation of the concentrations of the indicator species. It is, however, important to validate each new application so that side reactions (e.g. ion-pair association) of the chosen indicator are detected.

Table 4
Spectrophotometric determination of pH in Xylocaine® injections

Lidocaine–HCl (mg/ml)	pH	Mean difference (in pH units) between spectrophotometric and potentiometric methods	Number of samples
5*	6.76, 6.79	+0.045	2
10*	6.70–6.84	+0.056	5
20*	6.86–6.88	+0.081	7

*0.0185 M, 0.037 M and 0.074 M, respectively.
Temperature: 25°C.

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