Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 958–964

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Electrodeless, accurate pH determination in highly basic media using a new set of ^{1}H NMR pH indicators

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ARTICLE INFO

Article history:
Received 30 August 2010
Received in revised form
16 November 2010
Accepted 16 November 2010
Available online 25 November 2010

Keywords:
Highly basic media
NMR-pH indicator
Protonation constant
Electrodeless pH determination
Metformin

ABSTRACT

A set of indicator molecules was selected and applied to elaborate an NMR-based pH determination method, free of glass electrode errors in highly basic media. Accurate measurement of pH values and protonation constants was achieved by a successive build-up of overlapping, increasingly high pH solutions, using a collection of 8 compounds of appropriately incremented basicities.

In order to verify the method, acid–base properties were quantified for two compounds with very high basicities in conflicting reports: two pharmaceutically important biguanidine drugs, metformin and phenformin.

1. Introduction

The measurement of high pH and the concomitant determination of extreme basicities have always been a difficult task, as for highly basic solutions the response of the glass electrode deviates from the Nernstian behavior. This deviation adds a systematic error to the pH-meter readings, making the determined pH values incorrect. If the pH is high, the hydrogen ion activity is low; other cations (sodium, potassium, etc.) can replace the hydrogen ions in the gel layer of the glass membrane. As an apparent result a lower pH-value of the actual pH of the solution is measured. This phenomenon is the so-called alkaline error. It may also occur due to systematically changing junction potentials in line with the increasing contribution of the highly mobile OH^- ion, even in ionic strength adjusted alkaline solutions. Another difficulty about working with highly basic solutions is the extensive absorption of carbon dioxide, an obvious falsifying effect in standard buffer solutions, too.

To avoid this error several methods have been developed. The most recent IUPAC guidelines include the calculation of $[OH^-]$ and the isolation of samples from air [1]. The calibration of the glass electrode can be done in two ways: either by NIST standard buffers with dedicated pH values [2] or by titration of a strong acid with a strong base, where the pH is calculated for each measuring point, and it is compared to the potential of the electrode. This method can be automated by computer programs, which can estimate the alkaline error, the carbonate error and the ionic product of water [3,4]. By the different calibration methods one gets different pH scales: the first one results in the activity scale, where $pH = -\log a_{H^+}$, while the second one gives the concentration base pH-scale: $p[H] = -\log[H^+]$. Comparison of the two scales has been revisited [5].

pH can also be calculated from spectroscopic data, like UV–VIS absorbance [6] or chemical shifts in NMR [7–9].

NMR-pH measurements and titrations offer an alternative way. They have the advantage of monitoring several compounds in one single solution, and the accuracy of the pK_a determination is comparable or even superior of pH-potentiometric titrations. Popov et al. have published guidelines for the determination of high and low pK_a values [1]. These recommendations include the calculation of $[H^+]$ and $[OH^-]$, exclusion of D_2O as a solvent, the use of external reference and lock compounds and the complete isolation of the samples from air. Applying these suggestions makes the NMR-titrations more labour intensive and time consuming.

In the process of NMR-pH titration the pH-dependence of the chemical shift is observed. Since protonation is instantaneous on the NMR time scale, one common peak can be observed, which is the weighted chemical shift average of the protonated (δ_{HL}) and deprotonated (δ_L) forms. Weighting factors are molar fractions [10,11].

$$\delta^{obs} = \chi_{HL}\delta_{HL} + \chi_L\delta_L \quad (1)$$

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doi:10.1016/j.jpba.2010.11.022

The molar fractions can be expressed in terms of protonation constant K and $[H^+]$:

$$\chi_{HL} = \frac{[HL]}{[HL]+[L]} = \frac{K\left[H^+\right]}{1+K\left[H^+\right]} \quad (2)$$

Since $\chi_{HL} + \chi_L = 1$, Eqs. (1) and (2) can be combined:

$$\delta^{obs} = \frac{\delta_L + \delta_{HL}K\left[H^+\right]}{1+K\left[H^+\right]} = \frac{\delta_L + \delta_{HL} \times 10^{\log K - pH}}{1+10^{\log K - pH}} \quad (3)$$

According to Eq. (3), the limiting shifts (δ_L and δ_{HL}) and the protonation constant ($\log K$) can be obtained by nonlinear fitting. The accuracy is limited by the precision of the pH-measurement of the solution. Thus, if the traditional single-sample NMR titration with glass electrode pH-measurement is used, the $\log K$ value is restricted to be in the 1–12.5 range. With special care and attention $\log K$ values outside this region have been reported, such as debrisoquine (13.0), uracil (13.3), salicylic acid (13.3) or caffeine (0.6) [12,13].

If a monoprotic compound is used as an *in situ* pH indicator, the pH of the solution can be calculated from the observed chemical shift, by the rearrangement of Eq. (3):

$$pH = \log K_{ind} + \log\frac{\delta_{Ind}^{obs} - \delta_{HInd}}{\delta_{Ind} - \delta_{Ind}^{obs}} \quad (4)$$

Three series of *in situ* pH indicator molecules have so far been published by Szakács et al. [7], Tynkkynen et al. [8], and Baryshnikova et al. [9]. These molecules are suitable in acidic and neutral pH media, only HPO_4^{2-} is acceptable for basic solutions, which cannot be used for ^{1}H NMR titrations. It has therefore been necessary to compose a new series of ^{1}H NMR indicator molecules for basic solutions.

The main difficulty of determining the indicator parameters (δ_{Ind}, δ_{HInd}, and $\log K$) for compounds with large $\log K$ values is not just the precise calculation of the protonation constant, but also the determination of the limiting chemical shift of the deprotonated form (δ_{Ind}), which may even occur outside the pH-range.

An important advantage of NMR titration is that more than one compound can be measured simultaneously. Thus, the pH of the solution is necessarily identical. If a carefully chosen set of indicator molecules of gradually incremented, sufficiently close protonation constants is titrated, the indicator parameters can be determined sequentially:

$$pH = \log K_{Ind_1} + \log\frac{\delta_{Ind_1}^{obs} - \delta_{HInd_1}}{\delta_{Ind_1} - \delta_{Ind_1}^{obs}} = \log K_{Ind_2} + \log\frac{\delta_{Ind_2}^{obs} - \delta_{HInd_2}}{\delta_{Ind_2} - \delta_{Ind_2}^{obs}} \quad (5)$$

Perrin and Fabian [14] showed that relative protonation constants can be determined more precisely and accurately in a multicomponent NMR-pH titration than $\log K$ values themselves.

Rearranging Eq. (5), the indicator parameters of a molecule can be determined, if those of the other one are known and the difference between the $\log K$ values is small enough.

$$\delta_{Ind_2}^{obs} = \frac{\delta_{HInd_2}\left(\delta_{Ind_1}^{obs} - \delta_{Ind_1}\right) + 10^{\Delta\log K}\delta_{Ind_2}\left(\delta_{HInd_1} - \delta_{Ind_1}^{obs}\right)}{10^{\Delta\log K}\left(\delta_{HInd_1} - \delta_{Ind_1}^{obs}\right) + \left(\delta_{Ind_1}^{obs} - \delta_{Ind_1}\right)} \quad (6)$$

where $\Delta\log K = \log K_2 - \log K_1$

We have therefore selected an appropriate set of indicator molecules to cover the pH range 10–14. These molecules are small and NMR-wise simple. They have maximum two signals in their ^{1}H NMR spectra, they are inert towards other solution compounds and their protonation constants are close enough to build up a system of $\log K$ values determined precisely.

Based on these criteria a series of eight molecules was assembled: trimethylamine, sarcosine, *tert*-butylamine, 4-hydroxypyridine, cytosine, acetone oxime, acetamidine and methylguanidine (Fig. 1). Initial $\log K$ values were taken from [15].

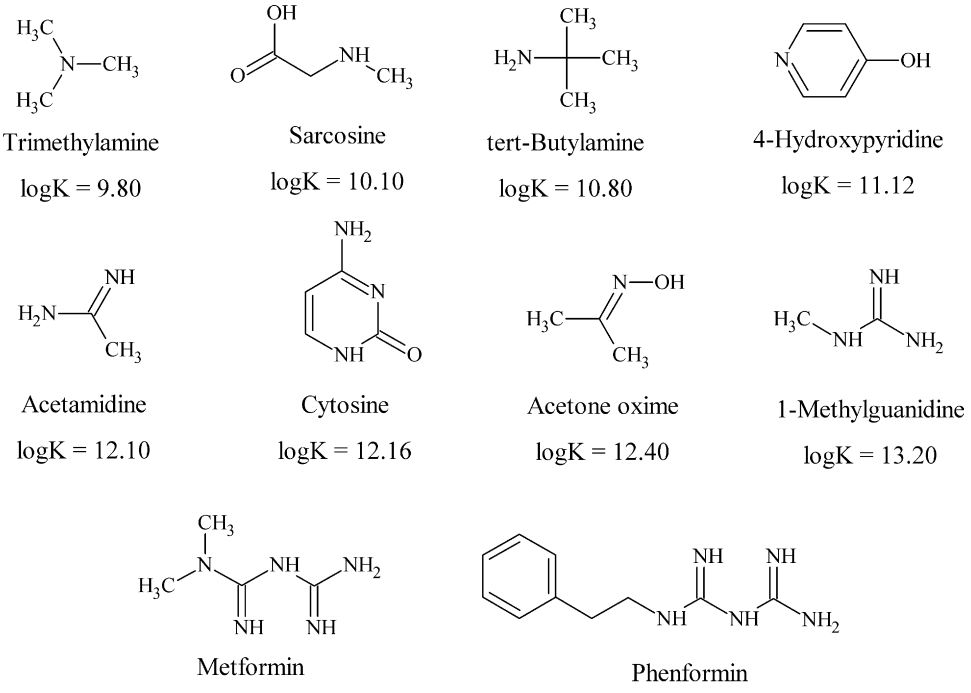

Fig. 1. Structures and literature protonation constants [15] of the indicator molecules and the biguanidine drugs.

In the literature, trimethylamine was the ^{1}H NMR indicator molecule of the largest log$K$ value [16], but it has been used at 0.15 M ionic strength, we therefore re-measured its indicator parameters at higher ionic strength.

2. Experimental

2.1. Chemicals

The indicators and other chemicals were purchased from Sigma–Aldrich. All chemicals were of analytical grade. The solutions were prepared with bidistilled water.

2.2. Determination of indicator parameters

Three stock solutions were prepared: 1 M NaOH, 1 M HCl and 2 M NaCl. Each solution contained 5% (v/v) D_2O, 0.1 mM sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS), 0.5 mM trimethylamine, 1 mM sarcosine, 0.5 mM *tert*-butylamine (TBA), 1 mM 4-hydroxypyridine, 1 mM cytosine, 1 mM acetone oxime, 1 mM acetamidine, 1 mM 1-methylguanidine.

The NaOH solution was titrated with equal volumes of the HCl and NaCl solutions to maintain the ionic strength at 1 M, and the pH was measured by a Metrohm 6.0234.110 glass electrode, which was calibrated according to the IUPAC recommendations [2], using 4 standard buffers: 0.05 M potassium tetraoxalate, 0.05 M potassium hydrogenphtalate, 0.025 M disodium hydrogenphosphate + 0.025 M potassium dihydrogenphosphate and 0.01 M sodium tetraborate. Temperature was kept at 25 ± 0.1 °C.

The small isotope effect of 5% D_2O was neglected, since it is within the deviation limit (0.02 pH units) according to the Gross–Butler–Purlee theory [17,18].

Measurements were carried out on a Varian Inova 600 MHz spectrometer equipped with a dual 5 mm inverse detection gradient probehead at 25 ± 0.1 °C. ^{1}H NMR spectra were recorded with double pulse field gradient spin echo pulse sequence [19] to suppress the solvent signal. Spectra were processed by VNMRJ 2.2C software.

2.3. Evaluation of the NMR titrations

Eq. (3) was fitted to the δ^{obs} versus pH datasets, and Eq. (6) was fitted to the δ_2^{obs} versus δ_1^{obs} datasets by the nonlinear fitting function of OriginPro 8 software [20], which allows the simultaneous fitting of multiple datasets.

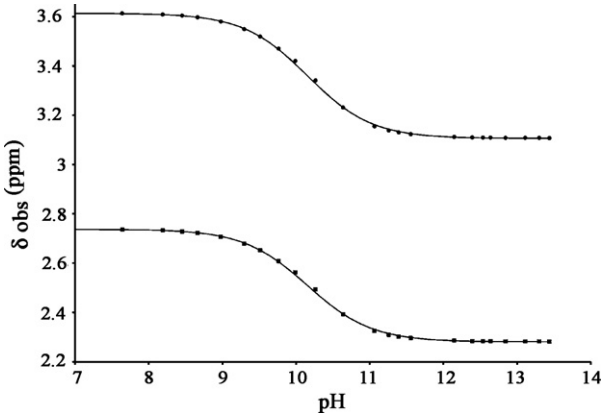

Fig. 3. ^{1}H NMR titration of sarcosine methyl (■) and methylene (●) protons. The computer fit is the solid line.

3. Results and discussion

3.1. Determination of the NMR indicator parameters

Trimethylamine is a suitable indicator molecule at 0.15 M ionic strength. If, however, the ionic strength is increased its signal intensively broadens, the line width is about 10–20 Hz, and it becomes asymmetric, which dramatically decreases the precision of chemical shift measurement (Fig. 2).

This phenomenon was not observed at any other molecules, so sarcosine was used as the most "acidic" indicator molecule, its indicator parameters were determined by plotting the measured chemical shifts against pH, determined by the glass electrode (Fig. 3). Note that the highest pH values are beyond 12, where the accuracy of the glass electrode is questionable, only the limiting chemical shifts have significance in this range. At pH near 10, where the accurate pH is important for the log$K$ determination, the glass electrode is accurate enough.

The resulting dissociation constant and limiting chemical shifts are: log$K$ = 10.15 ± 0.005, $\delta_L^{CH_2} = 3.106 \pm 0.001$ ppm, $\delta_{HL}^{CH_2} = 3.613 \pm 0.001$ ppm, $\delta_L^{CH_3} = 2.281 \pm 0.001$ ppm, $\delta_{HL}^{CH_3} = 2.736 \pm 0.001$ ppm. The indicator parameters of the other molecules were determined by fitting Eq. (6) to δ_2^{obs} versus δ_1^{obs} datasets. A typical plot of *tert*-butylamine versus sarcosine is shown in Fig. 4.

To check the accuracy and precision of the method mentioned above the indicator parameters of TBA were calculated on the basis of pH-meter readings, too. The results are shown in Table 1.

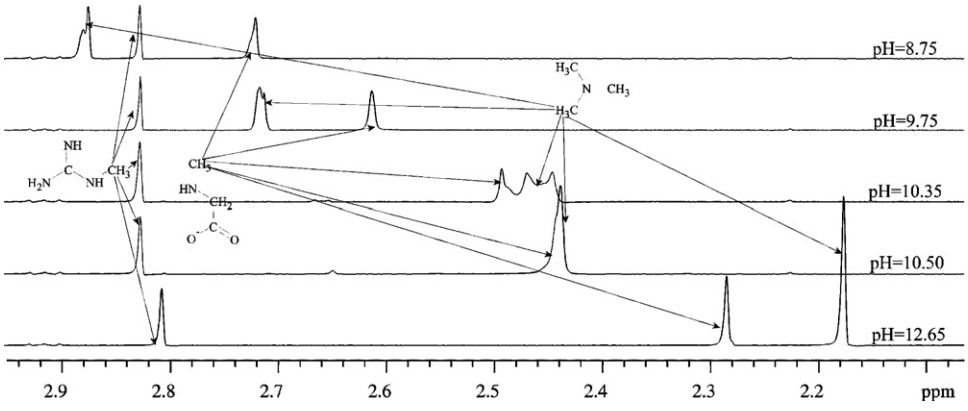

Fig. 2. Signal broadening of trimethylamine upon protonation.

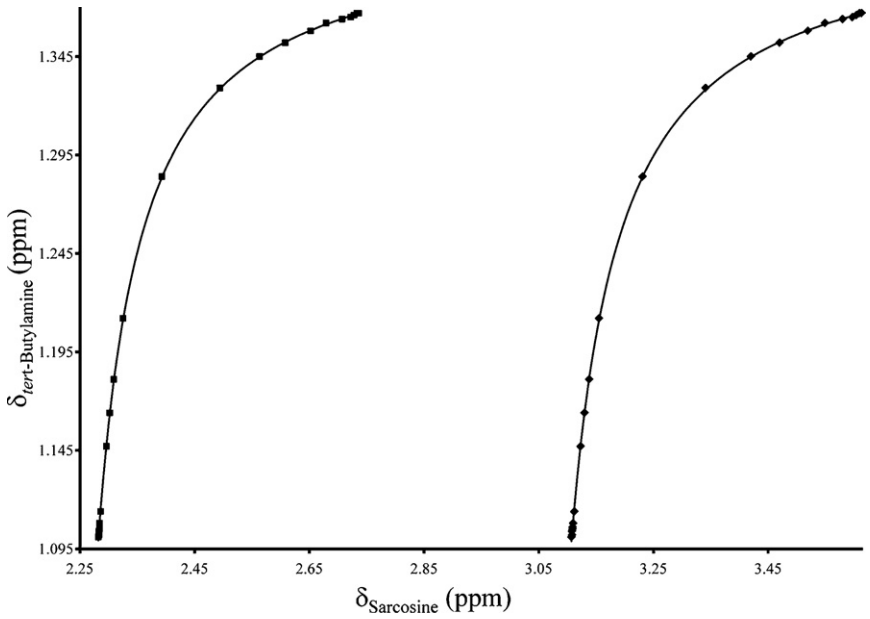

Fig. 4. Plot of ^{1}H chemical shift of *tert*-butylamine as a function of the chemical shifts of sarcosine. The solid line represents the computer fit.

Table 1
TBA parameters determined by the two methods.

	Perrin–Fabian method	pH-meter readings
$\log K$	10.99 ± 0.008	10.98 ± 0.006
δ_L	1.099 ± 0.001	1.097 ± 0.001
δ_{HL}	1.369 ± 0.001	1.368 ± 0.001

The indicator parameters of the other molecules were determined by the method of Perrin and Fabian. Acetamidine hydrolyzes rapidly at high pH. In an alkaline stock solution nearly 100% of acetamidine decomposes to acetamide and ammonia, by the time of the NMR experiment, precluding the measurement.

To handle this problem, 0.5 M acetamidine solution was prepared and 1 μl was transferred to each NMR tube just before recording the spectra. This makes possible to determine the parameters of methylguanidine, since the $\Delta\log K$ value between acetone oxime and acetamidine is too large for precise determination.

The indicator parameters are summarized in Table 2.

Interestingly, the protons of 4-hydroxypyridine and cytosine, in *meta* position from the deprotonating phenol group (at 8.0 and 7.7 ppm, respectively), show "wrong way shift", the chemical shift of the deprotonated form is higher than that of the protonated one (Fig. 5).

The pH of each solution was calculated according to Eq. (4), and it was compared to the pH readings (Fig. 6).

It can be seen that distortion of the glass electrode readings remains below 0.1 pH units up to pH = 13, but at higher pH, the difference grows dramatically, at 1 M NaOH it exceeds the 0.5 pH units.

The precision of the pH determination can be characterized by the quadratic rule of error propagation of Gauss [21], which has been applied by Szakács et al. [7] to calculate the total error of pH_{Ind}. The quadratic rule of error propagation is based on variances, which can be applied to Eq. (4), showing that the total variance can be calculated from the variances of four components:

$$\sigma_{pH}^2 = \left(\frac{\partial pH}{\partial \log K}\right)^2 \sigma_{\log K}^2 + \left(\frac{\partial pH}{\partial \delta_{Ind}^{obs}}\right)^2 \sigma_{\delta_{Ind}^{obs}}^2 + \left(\frac{\partial pH}{\partial \delta_{HInd}}\right)^2 \sigma_{\delta_{HInd}}^2 + \left(\frac{\partial pH}{\partial \delta_{Ind}}\right)^2 \sigma_{\delta_{Ind}}^2 \quad (7)$$

Eq. (7) can be rearranged to:

$$\sigma_{pH} = \sqrt{\sigma_{\log K}^2 + \frac{(\delta_{Ind} - \delta_{HInd})^2 \sigma_{\delta_{Ind}^{obs}}^2 + \left(\delta_{Ind}^{obs} - \delta_{HInd}\right)^2 \sigma_{\delta_{Ind}}^2 + \left(\delta_{Ind} - \delta_{Ind}^{obs}\right)^2 \sigma_{\delta_{HInd}}^2}{(\ln 10)^2 \left(\delta_{Ind}^{obs} - \delta_{HInd}\right)^2 \left(\delta_{Ind} - \delta_{Ind}^{obs}\right)^2}} \quad (8)$$

Table 2
The most important protonation and ^{1}H NMR chemical shift parameters and the useful pH-range of the indicator molecules.

	$\log K$	$\Delta\log K$	δ_L	δ_{HL}	pH-range
Sarcosine	10.15 ± 0.005		3.106 ± 0.001	3.613 ± 0.001	8.7–11.7
			2.281 ± 0.001	2.737 ± 0.001	
tert-Butylamine	10.99 ± 0.006	0.842 ± 0.002	1.099 ± 0.001	1.369 ± 0.001	9.7–12.3
4-Hydroxypyridine	11.09 ± 0.007	0.097 ± 0.004	7.992 ± 0.001	7.916 ± 0.001	10.4–11.8
			6.516 ± 0.001	6.602 ± 0.001	
Cytosine	11.98 ± 0.018	0.888 ± 0.017	7.750 ± 0.002	7.509 ± 0.001	11.2–12.7
			5.867 ± 0.002	5.992 ± 0.001	
Acetone oxime	12.08 ± 0.020	0.102 ± 0.006	1.828 ± 0.001	1.896 ± 0.001	11.5–12.7
			1.769 ± 0.001	1.897 ± 0.001	
Acetamidine	12.61 ± 0.021	0.530 ± 0.006	1.960 ± 0.001	2.232 ± 0.001	
1-Methylguanidine	13.43 ± 0.022	0.814 ± 0.004	2.691 ± 0.001	2.825 ± 0.001	>12.5

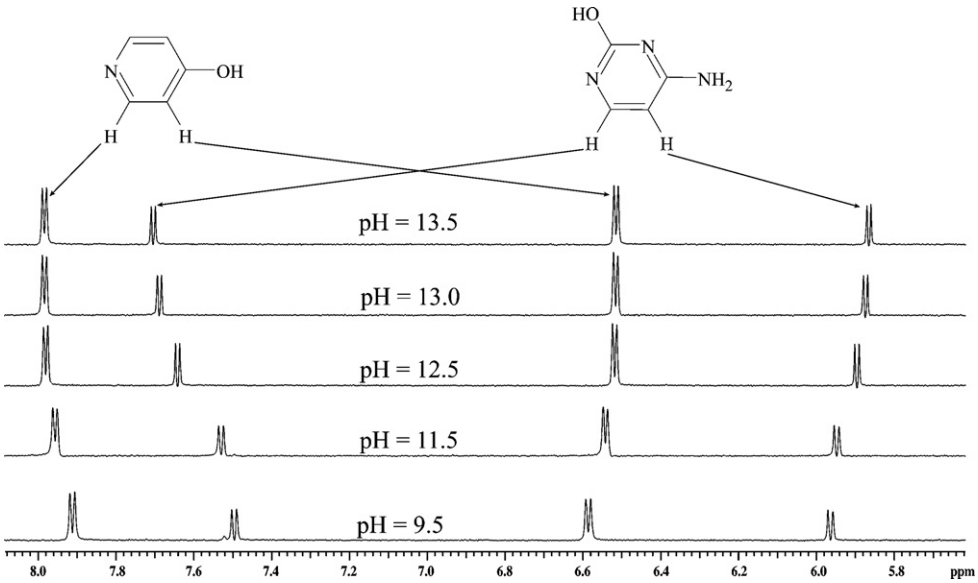

Fig. 5. Stackplot of the signals of 4-hydroxypyridine and cytosine.

Eq. (8) was used to characterize the uncertainty of pH-determination, using the variances of $\delta_{\mathrm{Ind}}^{\mathrm{obs}}$, δ_{HInd}, δ_{Ind} and $\log K$. The errors of the indicator parameters were taken from Table 2, whereas the uncertainty of the observed chemical shift was estimated to be 0.001 ppm, as reported by other authors [22].

From Fig. 7 the useful pH range of the indicators can be determined. Keeping the maximum errors at 0.05 pH units, the exact values can be determined and are listed in Table 2.

To prove the usefulness of the indicator molecules the protonation constants of metformin and phenformin, two extremely basic molecules were determined. Four new indicator molecules were used: sarcosine, *tert*-butylamine, acetone oxime and 1-methylguanidine. The pH values were calculated for the basic solutions (pH > 9), below that the pH-meter readings were used.

Metformin (1,1-dimethylbiguanide, Fig. 1) and phenformin (1-(2-phenylethyl)-biguanide, Fig. 1) are oral antidiabetics [23], although phenformin has been withdrawn from the market in 1978, because of the high incidence of lactic acidosis.

Biguanides are slightly weaker bases than guanidines, but they can be protonated twice. The second protonation step occurs in the acidic pH-region.

The protonation constants were determined by NMR-pH titrations. The protonation constants can be calculated by nonlinear fitting of the chemical shift versus pH. The observed shift is the weighted average of the three protonated forms (including the nonprotonated one):

$$\delta^{\mathrm{obs}} = \frac{\delta_{\mathrm{L}} + \delta_{\mathrm{HL}^+}\beta_1[\mathrm{H}^+] + \delta_{\mathrm{H_2L}^{2+}}\beta_2[\mathrm{H}^+]^2}{1 + \beta_1[\mathrm{H}^+] + \beta_2[\mathrm{H}^+]^2} \tag{9}$$

where β_1 and β_2 are the cumulative protonation constants, δ values are the chemical shifts of the different protonation forms.

Metformin has only one singlet in the ^{1}H NMR spectra, around 3 ppm, whereas phenformin has 2 triplets: around 3.5 ppm and 2.8 ppm and a multiplet at 7.3 ppm, but the triplet at 3.5 ppm is

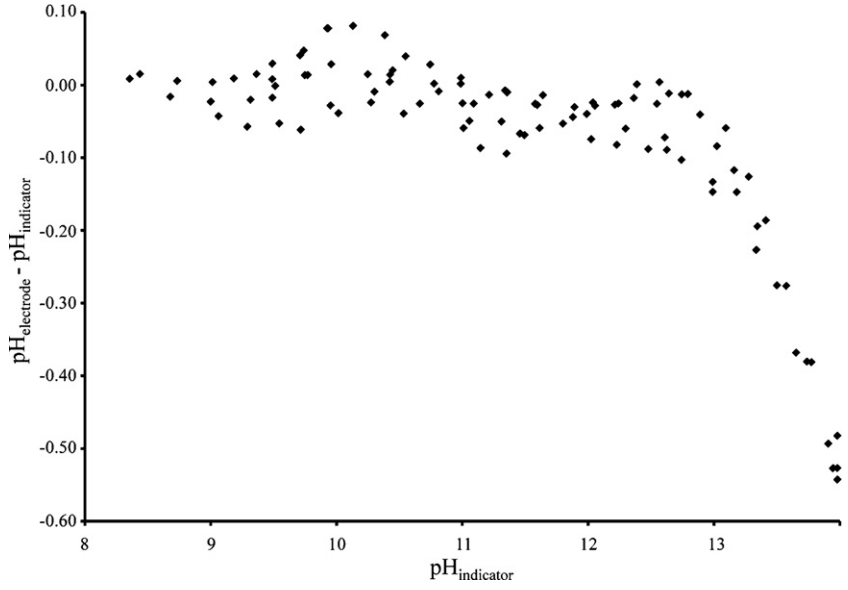

Fig. 6. The difference between the calculated pH and the pH meter readings.

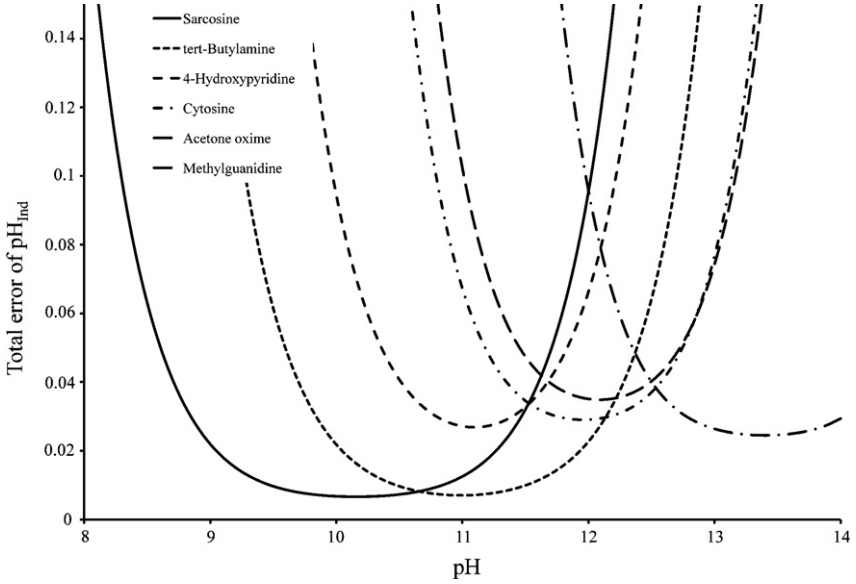

Fig. 7. The total error of pH determination by indicator molecules.

most sensitive of the protonation steps, this was the only one we used for the fitting procedures.

Both molecules are of extreme basicity; therefore the chemical shift of the deprotonated form cannot be measured at the applied ionic strength. Therefore, during the nonlinear fitting of Eq. (3) to the chemical shift versus pH datasets the $\log K$ and δ_L values will be strongly correlated, even if their standard deviation is acceptable, which questions the accuracy of the results.

Therefore the fitting procedure was done for several simulated δ^{obs} versus pH datasets, where the error of pH was ±0.05 units (the maximal error of the pH_{Ind} values), and the error of the chemical shift was ±0.002 ppm. The results showed that the accurate δ_L and $\log K$ values could only be calculated, if the last experimental pH was $\log K + 0.7$.

We also did the same simulations to validate the precision of the Perrin–Fabian method (Eq. (6)). Those results showed that the exact $\Delta\log K$ and δ_L could be calculated even if the protonation constant of the molecule is higher than the pH of the last experimental point.

The results could be conflicting at first sight, since Eq. (6) is the rearrangement of Eq. (3). This apparent discrepancy can be resolved, if we take into account the experimental errors: the standard error of pH determination is 0.05 pH units, while the accuracy of chemical shift determination is 0.002 ppm, at the most.

In our case, phenformin is less basic than methylguanidine, so the mostly basic solution (pH ≈ 14) must be at least 0.6 units higher than its protonation constant, so the fitting of Eq. (9) to the δ^{obs} versus pH dataset gives reliable $\log\beta$ values.

Metformin is a stronger base, thus, according to the simulations, its protonation constant cannot be precisely calculated by Eq. (9). $\log\beta_1$ could only be calculated with a standard error of 0.17, moreover, the correlation coefficient between $\log\beta_1$ and δ_L is −0.99 (Fig. 8A). It can also be seen, that the fitted curve has systematic error.

The limiting chemical shift can be calculated more accurately by the method of Perrin and Fabian, where the $\Delta\log K$ values are calculated from the chemical shifts of methylguanidine, which results in $\Delta\log K = 0.415 \pm 0.010$, and $\delta_L = 2.929 \pm 0.001$ ppm (Fig. 8B).

Since metformin can be protonated twice, Eq. (6) cannot be used to determine both $\log\beta$ values, so Eq. (9) was fitted to the δ^{obs} versus

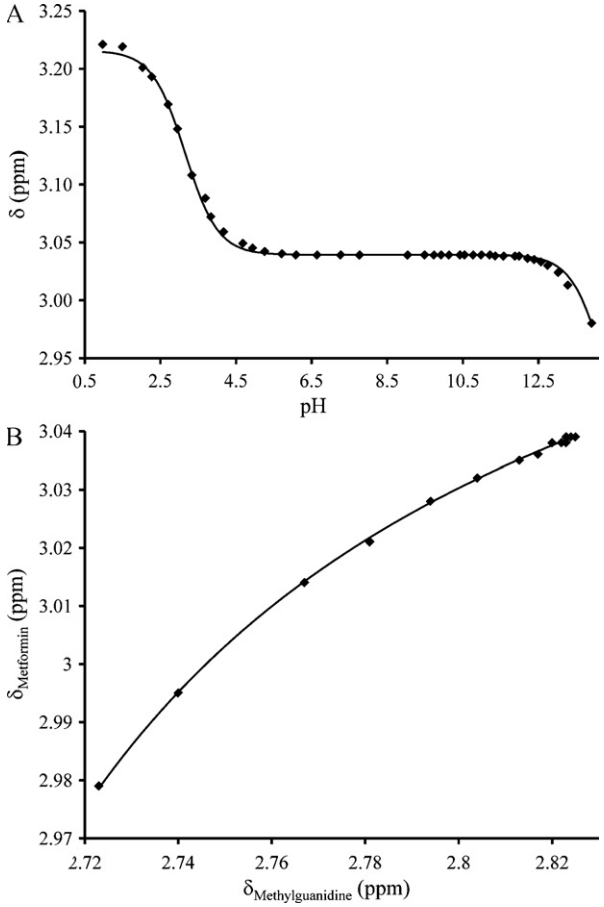

Fig. 8. The titration curves of metformin.

Table 3
The chemical shifts and protonation constants of metformin and phenformin.

	Phenformin	Metformin
$\log K_1$	13.27 ± 0.03	13.85 ± 0.03
$\log K_2$	3.26 ± 0.02	3.14 ± 0.02
δ_L (ppm)	3.366 ± 0.003	2.929 ± 0.001
δ_{HL^+} (ppm)	3.511 ± 0.001	3.040 ± 0.001
$\delta_{H_2L^{2+}}$ (ppm)	3.662 ± 0.001	3.216 ± 0.001

pH dataset, while δ_L was kept fixed during the nonlinear parameter fitting, so the protonation constants can be calculated more precisely, as shown in Table 3.

4. Conclusions

Both the accuracy and the precision of pH determination can be greatly improved by applying indicator molecules for highly basic media. These indicator molecules allow the precise determination of large log *K* values, such as polyamines and guanidines. As it was shown, the accuracy and precision of glass electrodes can be exceeded using *in situ* NMR-pH indicators, in particular methylguanidine at extremely high pH.

Our work is the first attempt to cover the basic pH range by ^{1}H-NMR indicators, as well as to quantitate the inaccuracy of the glass electrode at high pH levels. Compared to the guidelines of Popov et al. [1], our method is much faster and easier, since there is no need to calculate the hydroxide concentration, and one does not have to work under nitrogen or argon atmosphere. The indicator parameters listed in Table 2 can be used for the activity-based pH scale, at $t = 25\,^{\circ}C$ and 1 M ionic strength, but they can be used at any temperature and ionic strength, after measuring the parameters under those circumstances.

The indicator molecules were applied for *in situ* pH monitoring in the titration of metformin and phenformin. These indicator molecules were used to resolve the complete microspeciation scheme of the most natural amino acid, arginine [24].

Acknowledgement

This work was supported by OTKA T 73804 grant.

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