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Short communication

Potentiometric determination of ionisation constants for diphacinone and chlorophacinone in a dioxane–water cosolvent system

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ABSTRACT

The purpose of this study was to determine the ionisation constants of two poorly soluble compounds, namely diphacinone and chlorophacinone, potentiometrically in 1,4-dioxane–water mixtures with ibuprofen used as a standard. In this study, Gran's method was employed for the calibration of glass electrode in cosolvent systems with pH measurements based on the concentration scale (p_cH). Aqueous pK_a values for the tested compounds were obtained by extrapolation on a Yasuda–Shedlovsky plot. It was demonstrated that the pK_a for ibuprofen determined using this method was consistent with those reported in literature. The technique was applied successfully to the two indandione derivatives, diphacinone and chlorophacinone. The present study demonstrated that the use of an organic cosolvent is effective in improving the solubility of compounds allowing potentiometric determination of ionisation constants that are otherwise difficult in aqueous solutions.

1. Introduction

The extent of ionisation of weak acids and bases is governed by the pH of the medium and the tendency to which the compound ionises in an aqueous medium. The ionisation constant or pK_a, is an important parameter to formulation scientists to predict the site of dissolution and absorption for the drug.

Many new substances are poorly soluble in aqueous solutions, conventional potentiometric determination of ionisation constant of these compounds can often be difficult [1–6]. Other methods such as spectrophotometry [7–13] and capillary electrophoresis [14–16] provide an alternate for pK_a determination at concentrations as low as 20 and 50 μM, respectively [2,17]. For compounds that are practically insoluble in water (<1 μM), the approach of using an organic cosolvent allows investigators to determine the pK_a by potentiometric titration, which remains to be the method of choice for its effectiveness and reliability.

The use of cosolvent systems for the determination of ionisation constants was first introduced by Mizutani [18]. The use of mixtures containing 1,4-dioxane has also been reported previously [19–24]. Amongst the models developed to correlate the experimentally determined ionisation constants in cosolvent systems (p_sK_a) to aqueous pK_a values [18,25–28], the method described by Yasuda [29] and Shedlovsky and Kay [30] is perhaps the most widely accepted. The technique involves extrapolation of the p_sK_a values determined in cosolvent mixtures to the zero cosolvent value. Since the ionisation equilibrium of compounds in aqueous solution is not reflected in cosolvent system and that its activity is largely governed by the ratio of water content, it is therefore proposed by Yasuda and Shedlovsky that the plot of $p_sK_a + \log[H_2O]$ over its dielectric constant would yield a straight line. The Yasuda–Shedlovsky method is now widely used to determine ionisation constants of poorly soluble compounds [31–34].

Like many other drug compounds, first generation anticoagulants diphacinone (Fig. 1a) and chlorophacinone (Fig. 1b) are practically insoluble in water [35]. The present study was initiated by the lack of reliable literature reporting the ionisation constant for diphacinone, and while chlorophacinone was reported to possess a pK_a value of 3.40, there was no clear indication on the method used for the determination [36]. The present study aims to adopt the cosolvent method for potentiometric determination of pK_a values for both diphacinone and chlorophacinone. The ionisation constant of ibuprofen (Fig. 1c) was verified under the same conditions in order to validate the methods established in this present study.

2. Materials and methods

1,4-Dioxane (abbreviated to dioxane, ReagentPlus® ≥99% purity) and ibuprofen sodium (≥98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Diphacinone and chlorophacinone were provided by Dalian Experimental Chemical Co., Ltd. (Liao Ning, China).

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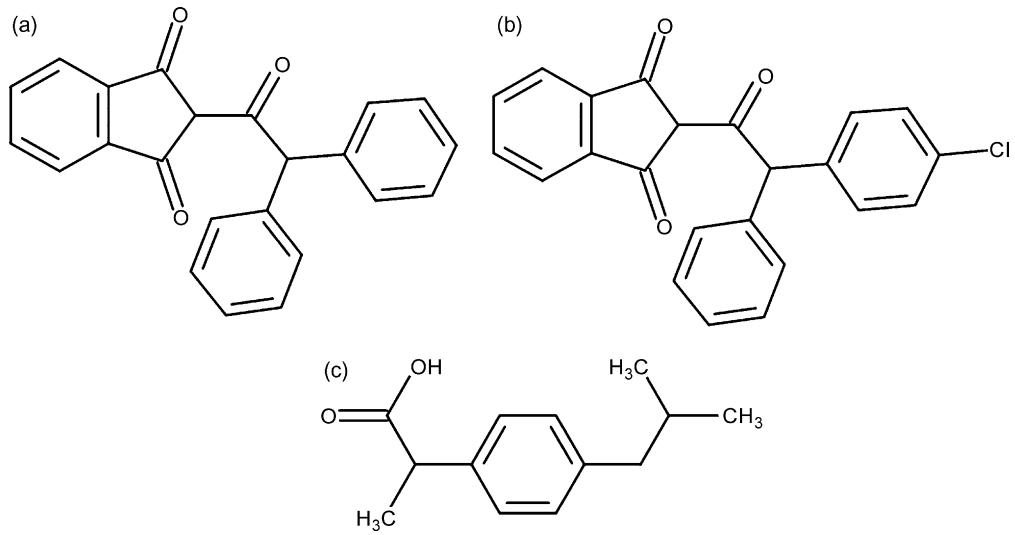

Fig. 1. Molecular structures of (a) diphacinone, (b) chlorophacinone, and (c) ibuprofen.

Carbon dioxide-free water used in all solvents was prepared by vigorous boiling of MilliQ water (resistivity ≥ 18 MΩ cm) for 20 min and then allowed to cool to room temperature under a stream of nitrogen before use. Potentiometric titrations were carried at 25 ± 1 °C with an InLab™ 413 glass pH electrode and SevenEasy™ pH meter (Mettler Toledo GmbH, Schwerzenbach, Switzerland).

Hydrochloric acid (0.2 M) and NaOH (0.2 M) were standardised against sodium carbonate (0.2 M) and potassium hydrogen phthalate (0.2 M), respectively. The primary standards were dried at 60 °C for 48 h before used and the standardisation procedure was performed six times in corresponding dioxane–water mixture for pK_a determination to ensure accuracy. All solutions were either freshly prepared or stored under a positive pressure of nitrogen in an amber plastic bottle. Stored solutions were purged with a stream of nitrogen gas for 15 min before reusing.

The dielectric constants for the dioxane–water cosolvent systems were compiled from various sources, and a regression correlation was established [37–40]. The resultant equation was used to calculate the dielectric constant for the solvent systems used in the present study (Eq. (1)):

$$y = 0.00005x^3 - 0.0039x^2 - 0.775x + 78.281 \ (R^2 = 0.9995) \quad (1)$$

where x is the ratio of dioxane in water and y is the corresponding dielectric constant (Fig. 2).

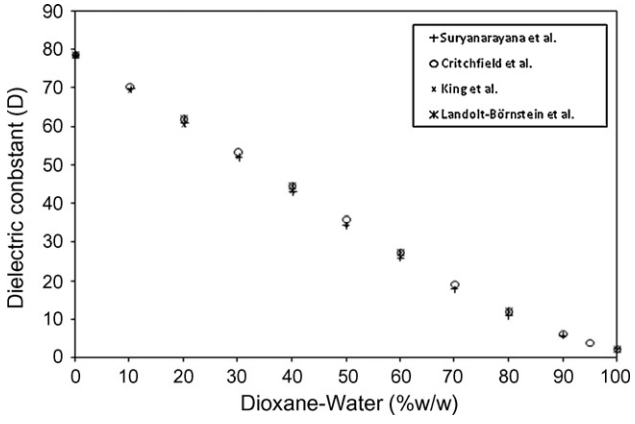

Fig. 2. Relationships between dielectric constant (D) and dioxane–water mixtures (% w/w).

The glass electrode was calibrated based on the Gran method. Dioxane–water mixtures containing 0.2 M HCl were calibrated with 0.2 M NaOH in the same dioxane–water concentration twice (I = 0.1). The GLEE computer program (Protonic Software, Berkeley, USA) utilising a non-linear least-square refinement was used to fit calibration to a modified Nernst equation (Eq. (2)) [41]:

$$p_cH = \frac{E - E_0}{s} \quad (2)$$

The standard potential (E_0) and the Nernstian slope (s) were used to correct the recorded values in millivolt (E) in the ^s_spH scale during titration into an operational pH (p$_c$H).

Dioxane–water mixtures containing 0.5–1.0 mM drug samples were titrated to approximately pH 12.0 with NaOH (0.2 M) using a micropipette. Ibuprofen was pre-acidified to approximately pH 3.0–4.0 before titrating alkalimetrically. Data was recorded in millivolts and change of every 2 pH U was recorded. After addition of the titrant, 30 s stabilisation of the reading was required before proceeding with the titration process. A propeller stirrer was placed in the titrand to induce mixing throughout the process. The p$_s K_a$ of sample solutions titrated in cosolvent systems is given by Eq. (3):

$$p_sK_a = p_cH - \log\frac{\alpha}{1-\alpha} - \log\frac{{}_s\gamma_{A^-}}{{}_s\gamma_{HA}} \quad (3)$$

where α is the fraction of drug reacted with the titrant; ${}_s\gamma_{A^-}$ and ${}_s\gamma_{HA}$ are the activity coefficients of the ionic and molecular species of the drug, respectively.

The Debye–Hückel theory [42] estimates the activity coefficient using the dielectric constant (D), temperature in Kelvin (T), the charge of the ion (z_i), the ionic strength (I), and the ionic diameter (a_i). The ionic diameter of a hydrated ion is a value between 1 and 10 Å and is generally unknown. As an alternative, the Güntelberg model [43] estimates the ionic diameter to be 3 Å, and the activity coefficient can be expressed as:

$$-\log\ {}_s\gamma_{A^-} = \frac{0.51 z_i^2 \sqrt{I}}{1 + \sqrt{I}} \quad (4)$$

By combining Eqs. (3) and (4), the modified equation can be written as:

$$p_sK_a = p_cH - \log\frac{\alpha}{1-\alpha} + \frac{0.51 z_i^2 \sqrt{I}}{1 + \sqrt{I}} \quad (5)$$

Table 1
Parameters of the Yasuda–Shedlovsky equation and p_sK_a values for the tested compounds in dioxane–water cosolvent systems.

Dioxane (% w/w)	Diphacinone		Chlorophacinone		Ibuprofen	
	p_sK_a [a]	$p_sK_a + \log[H_2O]$	p_sK_a [a]	$p_sK_a + \log[H_2O]$	p_sK_a [a]	$p_sK_a + \log[H_2O]$
30.00	3.83 ± 0.03	5.420	3.76 ± 0.03	5.346	5.16 ± 0.04	6.726
32.00	3.84 ± 0.07	5.418	3.77 ± 0.04	5.342	5.19 ± 0.02	6.769
35.00	3.85 ± 0.04	5.412	3.78 ± 0.02	5.339	5.23 ± 0.03	6.784
40.00	3.88 ± 0.01	5.406	3.81 ± 0.01	5.332	5.33 ± 0.01	6.850
R^2		0.9794		0.9807		0.9815

[a] p_sK_a values are presented as mean ± S.D. ($N = 3$).

Table 2
Extrapolated and literature aqueous pK_a values of tested compounds.

Drug compound	pK_a (extrapolated value)	pK_a (literature values)	Ionisation functional groups
Diphacinone	3.75	N/A	Enolic-hydroxyl
Chlorophacinone	3.67	3.40 [47]	Enolic-hydroxyl
Ibuprofen	4.47	4.31–4.91 [2,48–50]	Carboxyl

And the ionic strength is given by:

$$I = \frac{1}{2}\sum c_i z_i^2 \quad (6)$$

At least 8 pH measurements, from 20 to 80% of neutralisation ($\alpha = 0.2$–0.8), were made during the process of titration to determine the p_sK_a of both drugs in each cosolvent system. The pK_a value was obtained by the methods described by Yasuda–Shedlovsky on a plot of $p_sK_a + \log[H_2O]$ versus $100/D$ [25,44].

3. Results and discussions

Potentiometric titrations were performed in cosolvent systems comprising 30–40% (w/w) dioxane in water. The concentration of the organic content was determined from the basis that at least 30% (w/w) dioxane was required for diphacinone and chlorophacinone to dissolve and with the maximum content limited to dielectric constant >44. High concentration of dioxane (>40%) were not used for extrapolation due to increasing effects from interfering ions, such as sodium, potassium and chloride, which have significant interactions with the testing compound at reduced dielectric medium [31,45].

The parameters of the Yasuda–Shedlovsky equation are summarised in Table 1. Good linear correlation with regression coefficients (R^2) exceeding 0.97 was observed for all compounds tested in cosolvent systems comprising 30–40% (w/w) dioxane in water. The ionisation constants for diphacinone, chlorophacinone and ibuprofen were illustrated in the Yasuda–Shedlovsky plot in Fig. 3.

Fig. 3 shows the Yasuda–Shedlovsky extrapolations of diphacinone, chlorophacinone and ibuprofen with the pK_a values determined at zero dioxane content. The plots for diphacinone and chlorophacinone were characterised by slightly negative slopes contrary to a steeper positive slope for ibuprofen. The indandione compounds consist of a β-tricarbonyl system and can be perceived as ampholytes for their possession of acidic properties similar to diphenol compounds, and basic properties as the structure undergoes keto-enolic conversion [46]. Diphacinone and chlorophacinone have closely identical slopes that differ significantly from ibuprofen that possesses an acidic carboxyl functional group. The trends of slope polarity in relation to functional groups involved as observed in this study agrees with those reported in previous studies [32,33].

The ionisation constant for ibuprofen ($pK_a = 4.47$) determined in the present study showed good correlation with reported literature values (Table 2). The method was subsequently applied to the two

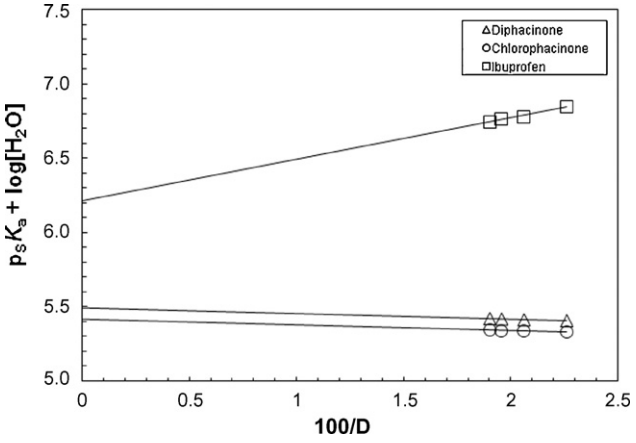

Fig. 3. The Yasuda–Shedlovsky plots for diphacinone, chlorophacinone and ibuprofen.

indandione compounds and their pK_a values were determined as 3.75 and 3.67 for diphacinone and chlorophacinone, respectively. The present work is the first to report the ionisation constant for diphacinone and the value determined for chlorophacinone is considered to be in a close range to the value of 3.40 reported by Tomlin [36]. The present study illustrated the methods for determining the pK_a for compounds with poor solubility by means of potentiometric titration using the extrapolation techniques described by previous authors on a Yasuda–Shedlovsky plot [25,44]. Furthermore, this study also successfully demonstrated the possibilities to perform electrode calibration and micro-titration with minimal laboratory equipments.

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