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High throughput method to characterize acid-base properties of insoluble drug candidates in water

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ABSTRACT

In drug design experimental characterization of acidic groups in candidate molecules is one of the more important steps prior to the in-vivo studies. Potentiometry combined with Yasuda-Shedlovsky extrapolation is one of the more important strategy to study drug candidates with low solubility in water, although, it requires a large number of sequences to determine pK_a values at different solvent-mixture compositions to, finally, obtain the pK_a in water ($^w_w pK_a$) by extrapolation. We have recently proposed a method which requires only two sequences of additions to study the effect of organic solvent content in liquid chromatography mobile phases on the acidity of the buffer compounds usually dissolved in it along wide ranges of compositions. In this work we propose to apply this method to study thermodynamic $^w_w pK_a$ of drug candidates with low solubilities in pure water. Using methanol/water solvent mixtures we study six pharmaceutical drugs at 25 °C. Four of them: ibuprofen, salicylic acid, atenolol and labetalol, were chosen as members of carboxylic, amine and phenol families, respectively. Since these compounds have known $^w_w pK_a$ values, they were used to validate the procedure, the accuracy of Yasuda-Shedlovsky and other empirical models to fit the behaviors, and to obtain $^w_w pK_a$ by extrapolation. Finally, the method is applied to determine unknown thermodynamic $^w_w pK_a$ values of two pharmaceutical drugs: atorvastatin calcium and the two dissociation constants of ethambutol. The procedure proved to be simple, very fast and accurate in all of the studied cases.

1. Introduction

The pK_a values, along with solubility, partition coefficients, and reaction rates, are the most important physicochemical properties of, either, active pharmaceutical ingredients and the excipients used to formulate pharmaceutical compounds. Specifically, the knowledge of the ionization state of a particular functional group is fundamental in order to understand the pharmacokinetic and pharmacodynamic properties of new drugs [1,2] The extent of ionization for a dissolved compound, which is a function of its intrinsic pK_a value(s) and of the medium pH, can determine its solubility, dissolution rate, reaction kinetics, complexation with drug carriers, transport across biological membranes, distribution to the site of action, renal elimination, metabolism, protein binding, or receptor interactions [3,4]. In addition, from dissociation constant data, the major species of pharmaceuticals as emerging pollutants present in the environment (usually in neutral pH range) can be estimated [5,6]

Clearly, research in many aspects of the drug sciences requires knowledge and use of drug water pK_a values to account physicochemical or biopharmaceutical meaningful results. Undoubtedly, those pK_a values should be independent of the experimental conditions used for the measurements. The main difficulty appears when the solubility in water is low, as it is often seen for drug-like molecules, which are often lipophilic to ensure adequate passive transfer across biological membranes. To circumvent this solubility problem, researchers have been using aqueous-organic co-solvent mixtures for pK_a determination. The apparent pK_a values that result from measurements in aqueous-organic solvent mixtures are then converted to the values that would result in pure water by means of mathematical extrapolations. Precision, however, will depend principally on: (i) the relationships raised between the apparent pK_a values and solvent composition and, (ii) the number of pK_a-composition data obtained for the fitting to the proposed model.

Several techniques devoted to pK_a determinations have been extensively discussed [7]. Most pK_a measurements on pharma-

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ceutical substances are based on relationships between the pH of the solution and a measured physicochemical quantity. The most widely used methods are based on classical potentiometric and conductometric techniques, or based on the indirect estimations of pK_a from fluorescence intensity and retention times in liquid chromatography [8] or from electrophoretic mobilities in capillary electrophoresis [9].

Potentiometric titration in aqueous solutions is a high-precision technique for the determination of the pK_a values of different substances. It is commonly used due its accuracy and the commercial availability of fast automated instruments. Classical potentiometric techniques have been successfully employed for pK_a determination of water-insoluble drugs, and even specific potentiometric equipments have been developed [10,11]. The method nowadays used to estimate aqueous pK_a requires numerous individual titrations to cover a wide range of solvent compositions at a constant temperature; and each one demanding the preparation of solutions of the electrolyte and the titrand at that specific solvent composition.

We have recently proposed an automated potentiometric method for obtaining multiple pK_a values of compounds over wide range of solvent compositions in a single automated sequential experiment [12,13]. This Fast Sequence Method (FSM) consists of measuring pH values of drug solutions under conditions in which only the organic-solvent content is systematically changed. Two solutions having exactly the same analytical molal concentrations of the components of the conjugated pair were prepared in two different co-solvent compositions. These two solutions were prepared weighing, first, the required amount of solid drug, adding then the required amount of hydrochloric acid or sodium hydroxide to obtain the desired ionization degree - e. g. 50.0% dissociated for the best buffer capacity composition-, adding then the required amount of water, and finally raising to the final volume with methanol. All solutions used in the preparation have known molarity, density and molality, all additions are done by weight, and solutions are raised to a final volume measuring the final weight, which allow to calculate the final density and composition of all components in all units. Thus, in the sequential determination, additions of small volumes of one of these solutions into an initial volume of the other solution allow a systematic variation of the solution potential as a consequence of solvation and the dielectric properties of the media. These mixture do not involve neutralizations of acidic groups of the target molecules with strong acids or bases, and we are not aiming to obtain an equivalence point or final point volume. Therefore, this procedure does not constitute a "titration". However, for the sake of simplicity and to follow the tradition, from now we will continue calling "titrant" to any solution dispensed from burette and "titrand" to the volume of solution which is set in the vessel at the beginning of each sequence. By changing the order between titrant and titrated solution the whole range between the initial compositions is covered. The FSM was accordingly applied to acquire more than 20 data (at a given temperature) in only 1 h. This denotes a significantly high throughput for the proposed method [11].

The aim of this work was to use the FSM for obtaining aqueous pK_a of poorly soluble substances by measuring pK_a in a huge number of co-solvent composition conditions by two single sequences. Measurements of dissociation constants of six pharmaceutical drugs having functional groups carboxylic, phenolic and (secondary) amine have been performed. Four of the drugs were chosen in the first part to validate the procedure. For this aim, pK_a of salicylic acid, ibuprofen, labetalol and atenolol were obtained by few FSM experiments along wide ranges of methanol/water composition and pK_a values in water are calculated by extrapolation. Results obtained are compared with those reported in the literature. Other empirical equations that could also be useful to describe the behavior of pK_a values at different solvent compositions are also evaluated. These expressions can use either, reciprocal dielectric constant ($1/\varepsilon$) or also the more direct form: solvent composition. Accuracy of the behaviors and extrapolations demonstrate the feasibility of using other more simple expressions to extrapolate pK_a values to that corresponding to pure water. Finally, the FSM is used to obtain pK_a of atorvastatin calcium and of ethambutol (pK_{a1} and pK_{a2}) at different compositions of methanol (MeOH)/water mixtures at 25.0 °C, and the obtained pK_a values are fit with the different expressions evaluated in the first part. Values in pure water are obtained. In this case, the lower limit in MeOH content can be determined by the solubility of the drugs, while the upper limit can be extended to MeOH amounts compatible with the range allowed by the glass electrode considering the prevention of glass membrane dehydration [14].

2. Experimental

2.1. Instrumentation

An automatic titrator, Schott TitroLine Alpha (Hofheim am Taunus, Germany) (20.000 ± 0.001 mL) with an Electrode Schott Blueline 11 pH (Vernon Hills, IL, EE. UU) was employed for pH measurements. A homemade glass cell with a "heart shape" with double inlet and conical bottom was constructed to allow the measurements of pH with the electrode immersed in a minimal initial volume (3.600 mL including the volume to host the magnetic stirrer) while keeping maximum total volume of about 20 mL (to allow the increasing volumes added). The initial volume was delivered with an automatic titrator Metrohm Titrino SM702 (Herisau, Switzerland)(3.600 ±0.001 mL). The cell was kept into a thermostatic bath (Lauda; Lauda-Königshofen, Germany) at 25.0 °C. The bath had a control of temperature of ±0.5 °C.

The titration device was controlled by computer through the serial port. The computer had a Linux OS (Xubuntu 8.10) installed and a homemade software application called Pytrator v1.0 programmed under Python. Sequences based on time intervals can be programmed specifying time, speed, and volumes dispensed by the automatic burette, power on/off for the water bath, power on/off for the magnetic stirrer in addition to acquisition and storage of potentiometric data.

2.2. Chemicals

Water was purified from a MilliQ® purification system (Simplicity, Millipore, Massachusetts, MA, USA). Methanol was HPLC grade and was purchased from Mallinckrodt (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). The chemical reagents used in this work were of analytical grade or better: hydrochloric acid 37% (w/w), sodium dihydrogen phosphate, disodium hydrogen phosphate were purchased from Merck (Darmstadt, Germany), potassium hydroxide from Cicarelli (Santa Fe, Argentina) potassium hydrogen phthalate from Fluka (Buchs, Switzerland) and Borax from Baker (Mexico DF, Mexico).

Pharmaceutical drugs standard labetalol ((RS)-2-hydroxy-5-{1-hydroxy-2-[(4-phenylbutan-2-yl)amino]ethyl}benzamide) and atenolol (>99%) ((RS)-2-{4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide) were obtained from Sigma Aldrich; salicylic acid (99.9%) from Baker, ibuprofen (99.9%) (2-[4-(2-methylpropyl)phenyl]propanoic acid), etambutol (99.87%) ((2S,2'S)-2,2'-(Ethane-1,2-diylbis(azanediyl))bis(butan-1-ol)) and calcium atorvastatin (97.49%) (calcium (3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) were kindly provided from the Drugs Production Unit (UPM) from Facultad de Ciencias Exactas at UNLP.

2.3. Solutions

For each drug, a pair of solutions with very different MeOH/water compositions was initially prepared. Each pair had exactly the same concentration of all the species, *i.e,* the same dissociation ratio (50%) between the components of the conjugated pair. All the components of the solutions were weighted and led to a final known volume in a calibrated flask; therefore, the molar, molal and the solute moles per kilogram of solution can be calculated (this last expression format for concentration facilitates the computation of each solvent composition). Hence, the degree of dissociation keeps constant over titration and, more important, the ionic strength can be easily calculated.

The lowest possible methanol content compatible with 1 mM solutions of each drug (without precipitation) was used for one extreme composition. Thus, some compounds could be measured from the water-rich region (*e.g.* labetalol), whereas the least soluble drugs examined here, ibuprofen and atorvastatin calcium, were measured from higher methanol concentrations (31%w/w for ibuprofen and 27%w/w for atorvastatin calcium). Following the same procedure, a second pair of solutions was prepared for each compound in order to obtain full duplicate sequences.

2.4. Measurement procedure

The control of relevant variables was critical to achieve high precision and accuracy in measured pK_a values. These variables include: pH meter calibration, temperature control, solvent composition, ionic strength, absence of atmospheric CO_2 contamination, and estimation methods for activity coefficients.

2.4.1. Calibration of the glass electrode

The glass pH electrode was calibrated by following the multipoint calibration procedure [15] using three aqueous standard solutions: potassium hydrogen phthalate, dihydrogen phosphate/hydrogen phosphate, and borax. The standard solutions were prepared according to the procedures recommended by IUPAC and NIST and were equilibrated at 25 °C before the measurements. An average of about ten potential readings was taken for each buffer calibration.

Since the glass membrane was exposed to substantial changes in solvent composition during the whole sequence, the electrode calibration was repeated after the sequence of additions had been completed to obtain an average calibration of the electrode response.

2.4.2. Fast sequence method

Two solutions with the same dissociation ratio and very different solvent compositions were used, one acting as the titrant and the other as the titrated solution. The procedure was performed in both directions exchanging titrant with titrated solutions.

The calibrated electrode was immersed into the solutions through a hole in the cap of the glass cell and the solutions were shaken to avoid changes in solvent compositions by condensation into the internal flask walls. Carbon dioxide was removed by flowing dried nitrogen in the cell head-space for a few seconds. After thermal equilibrium, an initial potential difference was measured. The syringe dispenser had been previously filled and purged with the titrand solution, the sequence of additions was started by adding a determined volume of solution from the dispenser. During one titration sequence, the volume of titrant added must be accurately measured in order to know the exact composition of the mixture. After each addition, a period of time was allowed for mixing and stabilizing the electrode potential before making a reading. The stabilization of the electrode response demanded a longer period as the amount of MeOH increased. The frequency of the additions was programmed in the software to take into account two different time periods according to the solvent content in the cell, *i. e.*, every 1 min for the water rich region and 3 min for the intermediate and high MeOH content mixtures. A complete set of compositions was obtained from both sequences including some overlapped region within the intermediate MeOH – composition range, because the sequences were programmed by keeping volume addition constant with a total volume of 20.000 mL burette.

The sequences programmed used for salicylic acid, ibuprofen, labetalol consisted of 20 additions, in the case of atenolol consisted of 15 additions while in the case of atorvastatin calcium and ethambutol consisted of 20 additions. Taking into account the experiments involves two sequences, one increasing and another decreasing solvent compositions, and it was done with duplicated pairs of solutions, therefore, sequences of 20 additions leaded to data sets of 80 values, sequences of 15 additions leaded to data sets of 60, and sequences of 10 leaded to data sets of 40 values.

Blank titration measurements were also developed to verify absence of acidity contribution from the solvents. The titration of blanks were conducted in solutions with the same ionic strength and temperature as those where pH measurements were carried out (I = 1 mM KCl and T = 25.0 ±0.5 °C).

2.4.3. Calculations

Regression and plots were done with QtiPlot v0.9.8.9 or SciDavis v1.D009, while other calculations where done with Calc v5.1.6.2 (LibreOffice 5).

3. Results and discussion

3.1. Determination of pH

The measurement of pH in solutions prepared in solvents different from pure water is not trivial. The calibration of the pH electrode can be made with buffer solutions prepared with exactly the same solvent as the one used for the solution to be measured. These pH values must be noted as (${}^{s}_{s}pH$ or ${}^{w}_{w}pH$ when the solvent is pure water) according to the IUPAC recommendations [15]. When the electrodes are calibrated with the aqueous buffer standards and then the pH is measured in solutions with a solvent different from water, the pH value obtained should be denoted as ${}^{s}_{w}pH$. The $\triangleq$-conversion parameter ($\triangleq = {}^{s}_{w}pH - {}^{s}_{s}pH$) relates both operational pH scales. This parameter depends on the solvent composition and temperature [16–18].

3.2. Data treatment

For a given dissociation equilibrium:

$$HA \rightleftarrows A + H$$

the thermodynamic dissociation constants (${}^{s}_{s}pK_a$) can be obtained from the equation:

$${}^{s}_{s}pK_a = -\log\left\{\frac{m_A + m_H - m_{OH}}{m_{HA} - m_H + m_{OH}}\right\} + \log {}^{s}_{s}\gamma_{HA} - \log {}^{s}_{s}\gamma_A + {}^{s}_{s}pH_m \quad (1)$$

where m are molal concentrations, and the subscript indicates the corresponding chemical species, basic (A) and acidic (HA) form of the conjugated pair. For simplicity, the corresponding charges on different species are omitted. The terms ${}^{s}_{s}\gamma_i$ and ${}^{s}_{s}pH_m$ denote the activity coefficients and pH in molal scales, respectively. Since all the solutions were prepared at relatively low concentrations, the contribution of hydrogen (for acidic compounds) and hydroxyl (for amines) ions in Eq. (1) cannot be neglected, and the known Henderson-Hasselbach considerations can not be applied. Activity coefficients of the neutral species were assigned a value of unity,

whereas the activity coefficients of the ions were estimated through the Debye–Hückel equation:

$$-\log {}^{s}_{s}\gamma_i = \frac{Az^2\sqrt{I}}{1 + a_0 B\sqrt{I}} \quad (2)$$

where I stands for the ionic strength in the molal scale, z is the charge of the ionic species, and A and a_oB are equation parameters that depend on the medium density and the dielectric constant. These parameters can be calculated by assuming the Bates–Guggenheim convention [19,20]:

$$A = 1.835x10^6 \sqrt{\frac{\rho_s}{(\varepsilon_s T)^3}} \quad (3)$$

$$a_0 B = 1.5 \left[\frac{\varepsilon_w}{\varepsilon_s}\frac{\rho_s}{\rho_w}\right]^{1/2} \quad (4)$$

where T is the absolute temperature and ε and ρ stand for the static dielectric constant and the density at T, respectively, and the subscripts w and s indicate pure water and the solvent (or solvent mixture), respectively [21]. The density and dielectric constants for solvent mixtures were obtained from [22]. The value of the ionic strength is needed in order to estimate the activity coefficient by means of Eq. (2); and since this calculation requires knowledge of the contribution of all the ionic species, iterative calculation was applied when required.

Finally, the values of ${}^{s}_{s}pH$are calculated by converting the experimental ${}^{s}_{w}pH$of the solutions by means of the $\doteq$-conversion parameter, taken from [18]. To simplify the calculations, experimental $\doteq$-data were fitted to an empirical equation:

$$\doteq = -9.2256 \times 10^{-6} \times^2 - 1.125.10^{-3}x + 0.9913 (R^2 = 0.99997) \quad (5)$$

where x is the composition in%w/w.

3.3. Validation step

The reliability of ${}^{w}_{w}pK_a$allowed by the combination of FSM + extrapolation is assessed by including in the experiments the determination of ${}^{s}_{s}pK_a$of compounds whose values had been reported by other authors and using more than one experimental method [23]. Thus, the acid dissociation constants (${}^{s}_{s}pK_a$) of four poorly water-soluble drugs with different functional groups: carboxylic (salicylic acid and ibuprofen), phenol (labetalol, ${}^{w}_{w}pK_a$) and amine (atenolol) were determined by the FSM method in MeOH–water mixtures, as it was described in a previous work where it was used for determining effect of acetonitrile on the pK_a of inorganic buffers. It must be remarked that all pK_a values obtained in this work are thermodynamic values and corresponds to I = 0. Yasuda [24] and Shedlovsky [25] independently derived the following correlation between ${}^{s}_{s}pK_a$ and the dielectric constant of the solvent at the given composition:

$${}^{s}_{s}pK_a + \log[H_2O] = A/\varepsilon + B \quad (6)$$

where $[H_2O]$ represents the molar water concentration and ε denotes the dielectric constant of the solvent mixture. The terms A and B are parameters of the regression. Yasuda–Shedlovsky expressions (from now Y-S equation) has been extensively used to assess acid dissociation constants in water/solvent mixtures of pharmaceutical substances with poor solubility in pure water [26–28]. Shedlovsky noted that, for the case of methanol/water mixtures, acceptable ${}^{w}_{w}pK_a$ (in pure water) can be obtained by extrapolation ${}^{s}_{s}pK_a$ values from solvent compositions below 65% by weight [26].

In Fig. 2 the results obtained in this work by means of the FSM for ibuprofen, salycilic, atenolol and labetalol, using methanol/water solvent mixtures are shown, on the base of the variables of the Yasuda and Shedlovsky. In plot a the ${}^{s}_{s}pK_a$+ log $[H_2O]$ of the two carboxylic acids can be seen as a function of $1/\varepsilon$, together with the Y-S regression indicated with solid line and the statistics indicated below. Evaluating the Y-S line at x-point corresponding to the reciprocal static dielectric constant of pure water at 25 °C (ε = 78.5), $1/\varepsilon$ = 0.01274, leads to ${}^{s}_{s}pK_a$+ log $[H_2O]$ = ${}^{w}_{w}pK_a$+ 1.743, from where ${}^{w}_{w}pK_a$ can be obtained. This extrapolation value in the $(1/\varepsilon)$-axis of plot a is the lower point of this x-axis, and is the same criterion used in all the plots of this work using $1/\varepsilon$–scale. Plot b in the same figure shows the residuals of the Y-S regression, given as ${}^{s}_{s}pK_a$ differences between the values predicted by the regression and these obtained experimentally. Homogeneous distribution of the residuals indicate a good fit of the Y-S equation to experimental data.

Following the same working line, FSM was applied to study other two compounds: atenolol as a member of the amine family, and the dissociation constant of the phenolic hydroxyl group of labetalol. Results are depicted in plot c of the same figure, indicating again with continuous line the Y-S regressions. However, in this case, a difference can be clearly noted. Despite the suggestion of Shedlovsky about extrapolate from methanol compositions below 65% in this work we used FSM to obtain ${}^{s}_{s}pK_a$ along an extended solvent composition ranges. The dissociation equilibrium of labetalol, the phenol, as also in dissociations of carboxylic acids, involves the dissociation of a neutral molecule to generate two charged species. In all these cases the Y-S behavior is quite linear, even beyond 84% by weight. Plot d of Fig. 1 shows residuals of these Y-S regressions showing a proper fit. However, in the case of atenolol, the amine, whose dissociation involves charged species on both sides of the equilibrium, the behavior deviates from the linear Y-S at methanol compositions higher than 65%. Y-S regression depicted with solid line for atenolol in plot c of Fig. 1, residuals shown in plot d and parameters reported in Table 1 are obtained by linear regression of values from 14% to 65% by weight.

The results of all ${}^{w}_{w}pK_a$ obtained by Y-S extrapolations for the four compounds mentioned above are listed also in Table 1 together with several ${}^{w}_{w}pK_a$ values compiled from literature. ${}^{w}_{w}pK_a$ values reported by other authors, determined in a variety of techniques and conditions, show good agreement with the values obtained in this work by FSM and Y-S extrapolation, which validate the hereby proposed procedure. The only ${}^{w}_{w}pK_a$ value that could be noted higher than most of the data values found in literature is that obtained for ibuprofen. However, it must be noted that all reported values for this compounds are given for ionic strengths different than zero, excepting one of them which is only 0.17 lower than our value. Yasuda-Shedlovsky extrapolations give satisfactory results, but it is not infallible [26]: it has been reported [11], that extrapolation plots including the ${}^{s}_{s}pK_a$ values from solvent rich compositions usually exhibits sub-linear behavior, in general when extrapolations derives from higher solvent compositions. Greater differences can be found depending on from how far the ${}^{w}_{w}pK_a$ values are extrapolated from the experimental data.

For the aim of exploring alternative regressions the same ${}^{s}_{s}pK_a$ values are fit to different expressions and/or using different variables, and ${}^{w}_{w}pK_a$ are calculated by extrapolation of them. The chosen variables are reciprocal dielectric constant, as it is usually done in Yasuda-Shedlovsky extrapolations, but also in percent by weight of organic solvent, which can result more simple and easy to use. The following relationships are evaluated:

$${}^{s}_{s}pK_a = y_0 + (A\ w)\ \exp(B\ w) \quad (7)$$

where y_0, A and B are parameters of the equations, and w indicates organic solvent in percent by weight.

$${}^{s}_{s}pK_a + \log[H_20] = y_0 + (A\ w)\ \exp(B\ w) \quad (8)$$

using the same symbols as in previous equations

$${}^{s}_{s}pK_a = y_0 + A/\varepsilon \quad (9)$$

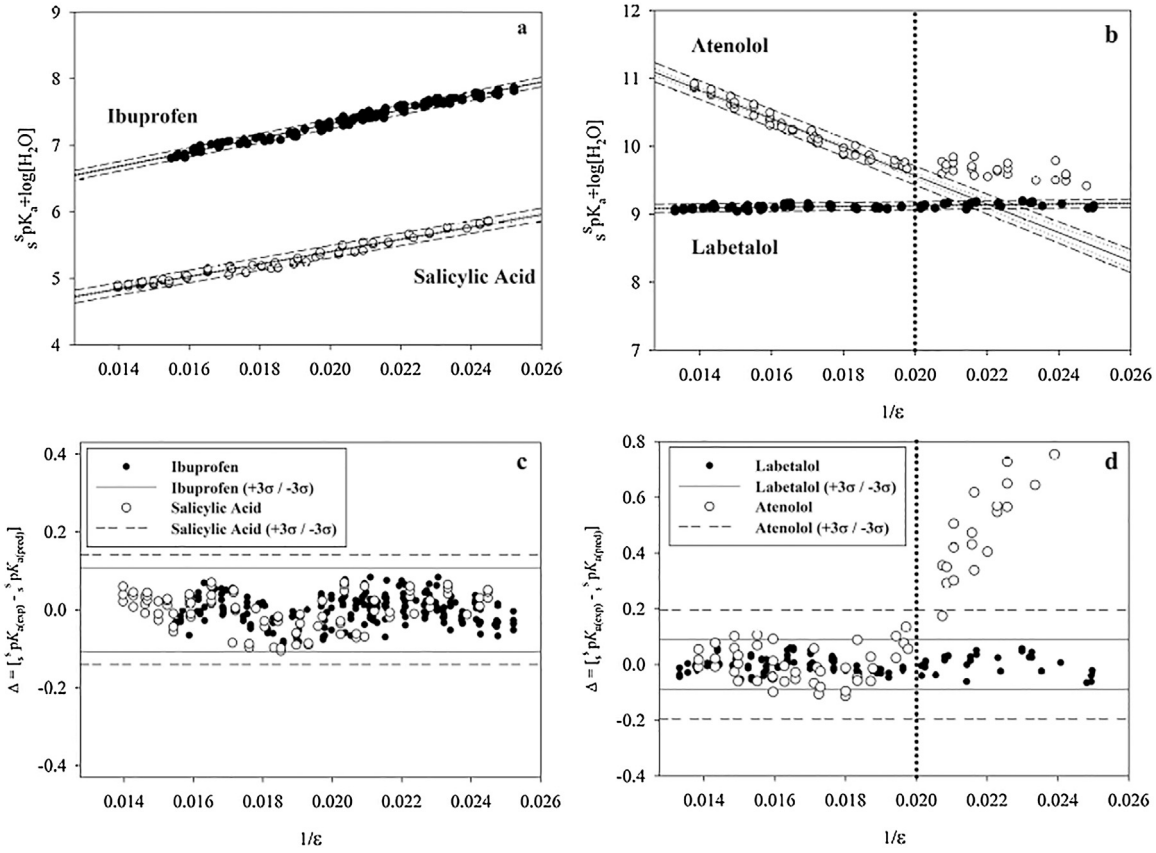

Fig. 1. Yasuda-Shedlovsky regressions for ibuprofen, salicylic acid, atenolol and labetalol. Plots a and b Yasuda-Shedlosvski regressions indicating with solid line the values predicted by the regression, dashed lines the 95% prediction band and dotted line the confidence interval. x-axis scale starts in $1/\varepsilon$ value corresponding to pure water at 25 °C ($78.54^{-1} = 0.01277$). Plots c and d, residuals of the regressions, given as ${}^{s}_{s}pK_a$ differences between experimental values and the values predicted by the regression, as a function of $1/\varepsilon$.

Fig. 2 show plots of: (a).- ${}^{s}_{s}pK_a$ *vs.*%w/w, (b).-${}^{s}_{s}pK_a$ + log[H_2O] *vs.*%w/w, (c).- ${}^{s}_{s}pK_a$ *vs.* $1/\varepsilon$ for the four reference compounds, fitting the relationships 7, 8 or 9 in the respective plots. Parameters resulting of the regressions are gathered in Table 2. In order to facilitate the comparison, results of Y-S regressions are repeated in the last row of each compound. A comparison of the plots in Fig. 2 makes evident that behaviors of carboxylic acids are not linear when the variable is%w/w, either when the function is ${}^{s}_{s}pK_a$ or either when it is (${}^{s}_{s}pK_a$ + log[H_2O]). On the contrary, it is very linear in any case where the variable is $1/\varepsilon$. In the case of labetalol, the phenol, the behavior is also very linear in all the cases, using all variables and all functions in the full range of compositions. On the contrary, atenolol, whose behavior was far from linear behavior in Y-S plots of Fig. 3 at methanol contents higher than 65% by weight (or $1/\varepsilon > 0.020$), now is also far from linear in plot c of Fig. 4 when the variable is $1/\varepsilon$. It is neither linear in the scale of weight percent when the function ${}^{s}_{s}pK_a$. However, using weight percent as variable and ${}^{s}_{s}pK_a$+ log[H_2O] gives a very linear behavior for atenolol even beyond 65% by weight.

Accordingly to the aforementioned analysis, the combination given by Eq. (8) and depicted in plot b of Fig. 4 result the best to describe the behavior dissociation constants of the studied chemical groups. Further studies, of more compounds along wide ranges of solvent compositions, would provide a solid base to give a more general conclusion about the linear behavior solvent composition range and extrapolation threshold. Until these amount of values are not available the analysts must apply the method to obtain ${}^{s}_{s}pK_a$ along the wider possible range of solvent compositions to, finally chose a proper solvent composition range to perform the extrapolation.

Beyond linearity is a desired property aiming to describe the behavior of ${}^{s}_{s}pK_a$ as a certain function of solvent composition, the extrapolation of values up to methanol 0% or $1/\varepsilon = 1/78.5$ (0.01274) can be more or less accurate ${}^{w}_{w}pK_a$. In Table 2 ${}^{w}_{w}pK_a$ values obtained with each expression are gathered, while values from literature can be recalled from Table 1. In general, an agreement can be observed between expressions depending on $1/\varepsilon$. which leads, in the case of both carboxylic acids and the phenol group compound, to values ~0.2 units below than the values obtained with expressions depending on percent by weight.

Comparing ${}^{w}_{w}pK_a$ obtained with all mathematical expressions with the values reported in literature suggest than the values obtained here with all models are slightly higher than the cloud of literature values in the case of carboxylic acids, slightly lower in the case of phenols. However, since reported values have been calculated for a wide variety of ionic strength conditions with few exceptions, this rough analysis can not be considered as conclusive.

In the case of labetalol, model of amine group, ${}^{w}_{w}pK_a$ obtained by all extrapolations showed excellent agreement between them and also with the literature values. It must be remarked for this case that only dependency given by Eq. (8) allow to extrapolate from methanol compositions higher than 65% by weight, which suggest to be a promising choice for the case of drug candidates with very low solubilities.

3.4. Determination of ${}^{w}_{w}pK_a$ values of atorvastatin and ethambutol

The automated potentiometric method has also been applied to determine the ${}^{w}_{w}pK_a$ values of atorvastatin calcium and of etham-

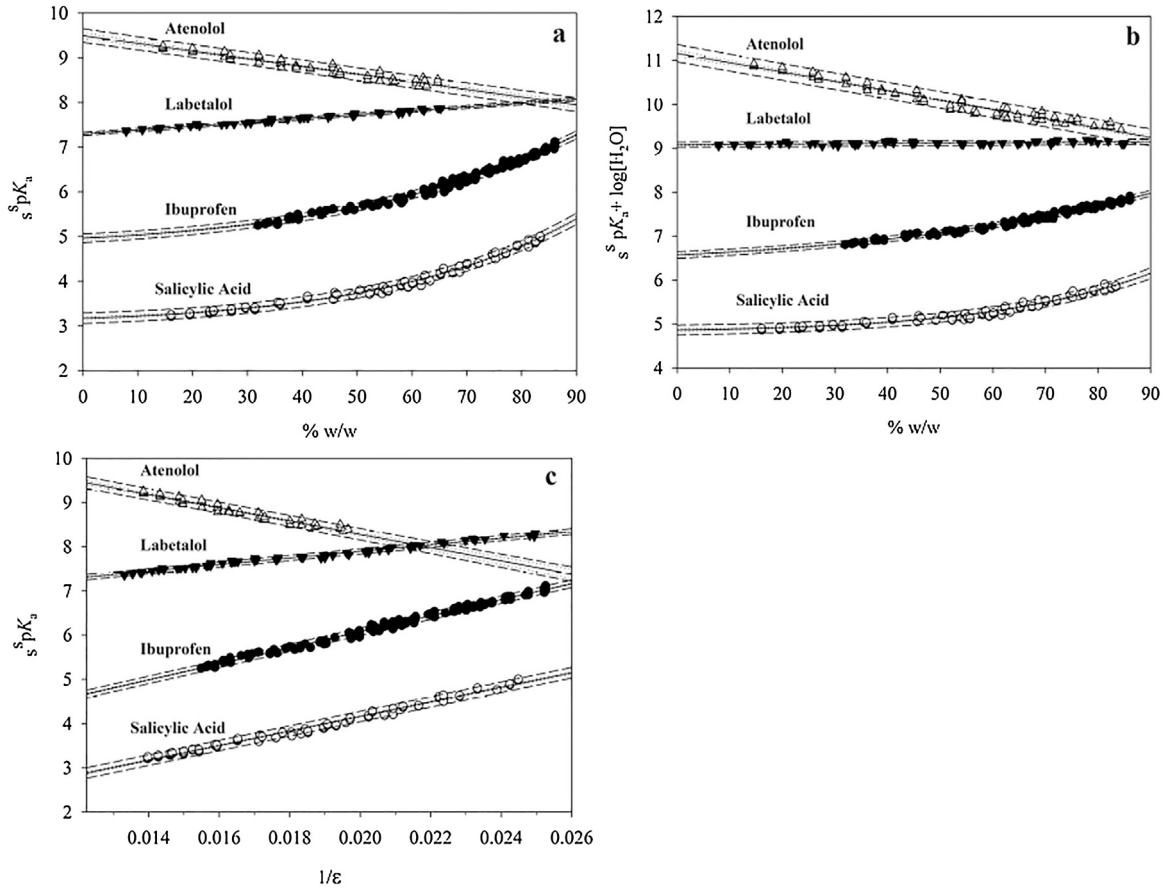

Fig. 2. Empirical regressions of ${}^{s}_{s}pK_a$ values for ibuprofen (●), salicylic acid (○), labetalol (▼) and atenolol (Δ). Solid lines represent the regressions given as: Plot (a) ${}^{s}_{s}pK_a$ vs.%w/w, Plot (b) (${}^{s}_{s}pK_a$ + log[H_2O]) vs%w/w, and Plot (c) ${}^{s}_{s}pK_a$ vs. 1/ε. Segmented line represent the 95% prediction bands, and dotted lines the confidence intervals.

Table 1
Parameters and Results obtained from Yasuda-Shedlovsky fits of experimental ${}^{s}_{s}pK_a$ to solvent mixture composition accordingly to Eq. (6) a for ibuprofen, salicylic acid, atenolol and labetalol. ${}^{w}_{w}pK_a$ indicates values obtained by extrapolation and ${}^{w}_{w}pK_{a\ \text{literature}}$ are values found in the Reference of last column. S. D. of each value is indicated in parentheses.

Compound	A	B	r^2	${}^{w}_{w}pK_a$ [1]	${}^{w}_{w}pK_{a\text{Literature}}$	Ref[2]
Ibuprofen	105.8 (0.9)	5.20 (0.02)	0.99	4.81 (0.04)	4.30–5.16[a] (I = 0.15 M, 25 °C)	[S1]
					4.45 (0.04)[a] (I = 0.15 M, 25 °C)	[S2]
					4.51 (0.07)[a] (I = 0.1 M, 25 °C)	[S3]
					4.5–4.6[a]	[S4]
					4.64 (0.04)[a] (I = 0, 25 °C)	[S5]
					5.2[a]	[S6]
Salicylic acid	92.9 (1.6)	3.54 (0.03)	0.95	2.99 (0.05)	2.54 (0.05)[a] (I = 0.15 M, 25 °C)	[S7]
					2.79 (0.01)[a] (I = 0.15 M, 25 °C)	[S7]
					2.83 (0.03) [a] (I = 0.5 M, 25 °C)	[S8]
					2.95[a] (23 °C)	[S10]
					2.99[b] (I = 0.15 M, 25 °C)	[S11]
					3.52[c] (I = 0.01 M, 25 °C)	[S12]
Atenolol	−206 (5)	13.71 (0.09)	0.97	9.32 (0.08)	9.25[a] (I = 0.15 M, 37 °C)	[S13]
					9.4[a] (I = 0.1 M, 25 °C)	[S14]
					9.54 (0.01)[a] (I = 0.15 M, 25 °C)	[S15]
					9.56[a] (I = 0.15 M, 25 °C)	[S16]
					9.60[b] (21–24 °C)	[S17]
Labetalol	5.7 (0.9)	9.01 (0.02)	0.26	7.34 (0.03)	7.32[d]	[S19]
					7.35[a] (I = 0.1 M, 25 °C)	[S14]
					7.35 (0.03)[b] (I = 0.05 M,20–25 °C)	[S20]
					7.41 (0.01)[e] (I = 0.15 M, 25 °C)	[S20]
					7.44[a] (25 °C)	[S21]
					7.5 (0.1)[f] (I = 0.15 M, 25 °C)	[S20]

[1] Thermodynamic values (I = 0).
[2] Reference list given as Supplementary Material.
[a] Potentiometric.
[b] CE/pH.
[c] HPLC.
[d] COSMOS-RS.
[e] D-PASa.
[f] Traditional UV/pH.

Table 2
Parameters and Results obtained from fits of experimental ${}^{s}_{s}pK_a$ to solvent mixture composition properties for ibuprofen, salicylic acid, atenolol and labetalol at 25 °C. S. D. of each value indicated in parentheses.

Compound	Regression	y_o	A	B	${}^{w}_{w}pK_a$	r^{2} [d]
Ibuprofen	${}^{s}_{s}pK_a$ *vs.* %w/w[a]	4.96 (0.02)	60×10^{-4} (4×10^{-4})	15×10^{-3} (7×10^{-4})	4.96 (0.05)	0.99
	${}^{s}_{s}pK_a$+ log[H_2O] *vs* %w/w[a]	6.57 (0.07)	60×10^{-4} (5×10^{-4})	11×10^{-3} (8×10^{-4})	4.82 (0.04)	0.99
	${}^{s}_{s}pK_a$*vs* 1/ε[b]	2.45 (0.02)	181 (1)	–	4.76 (0.05)	0.99
	${}^{s}_{s}pK_a$+ log [H_2O] *vs* 1/ε[c]	–	105.8 (0.9)	5.20 (0.02)	4.81 (0.04)	0.99
Salicylic acid	${}^{s}_{s}pK_a$ *vs.* %w/w[a]	3.17 (0.02)	4×10^{-4} (0.4×10^{-4})	20×10^{-3} (10^{-3})	3.17 (0.04)	0.99
	${}^{s}_{s}pK_a$+ log [H_2O] *vs* %w/w[a]	4.86 (0.02)	20×10^{-4} (3×10^{-4})	2.3×10^{-2} (2×10^{-3})	3.12 (0.04	0.96
	${}^{s}_{s}pK_a$ *vs* 1/ε[b]	0.87 (0.04)	165 (2)	–	2.97 (0.07)	0.99
	${}^{s}_{s}pK_a$+ log [H_2O] *vs* 1/ε[c]	–	92.9 (1.6)	3.54 (0.03)	2.99 (0.05)	0.95
Atenolol	${}^{s}_{s}pK_a$ *vs.* %w/w[b]	9.50 (0.02)	-18×10^{-3} (5×10^{-4})	–	9.50 (0.05)	0.96
	${}^{s}_{s}pK_a$+ log[H_2O] *vs* %w/w[b]	11.16 (0.03)	-21×10^{-3} (6×10^{-4})	–	9.42 (0.06)	0.95
	${}^{s}_{s}pK_a$ *vs* 1/ε[b]	11.28 (0.09)	−150 (5)	–	9.37 (0.07)	0.95
	${}^{s}_{s}pK_a$+ log [H_2O] *vs* 1/ε[c]	–	−206 (5)	13.71 (0.09)	9.32 (0.08)	0.97
Labetalol	${}^{s}_{s}pK_a$ *vs.* %w/w[b]	7.295 (0.005)	9.0×10^{-3} (0.1×10^{-3})	–	7.30 (0.02)	0.98
	${}^{s}_{s}pK_a$+ log[H_2O] *vs* %w/w[b]	9.076 (0.007)	8×10^{-4} (1×10^{-4})	–	7.33 (0.02)	0.98
	${}^{s}_{s}pK_a$ *vs* 1/ε [b]	6.39 (0.02)	74.9 (0.9)	–	7.33 (0.03)	0.98
	${}^{s}_{s}pK_a$+ log [H_2O] *vs* 1/ε[c]	–	5.7 (0.9)	9.01 (0.02)	7.34 (0.03)	0.26

[a] Data fitted to the empirical equation: f = y_0+x A.*exp*(Bx).
[b] Data fitted to f = y_0 + A.x.
[c] Data fitted to Yasuda-Shedlovsky expression.
[d] r^2 denotes the correlation factors.

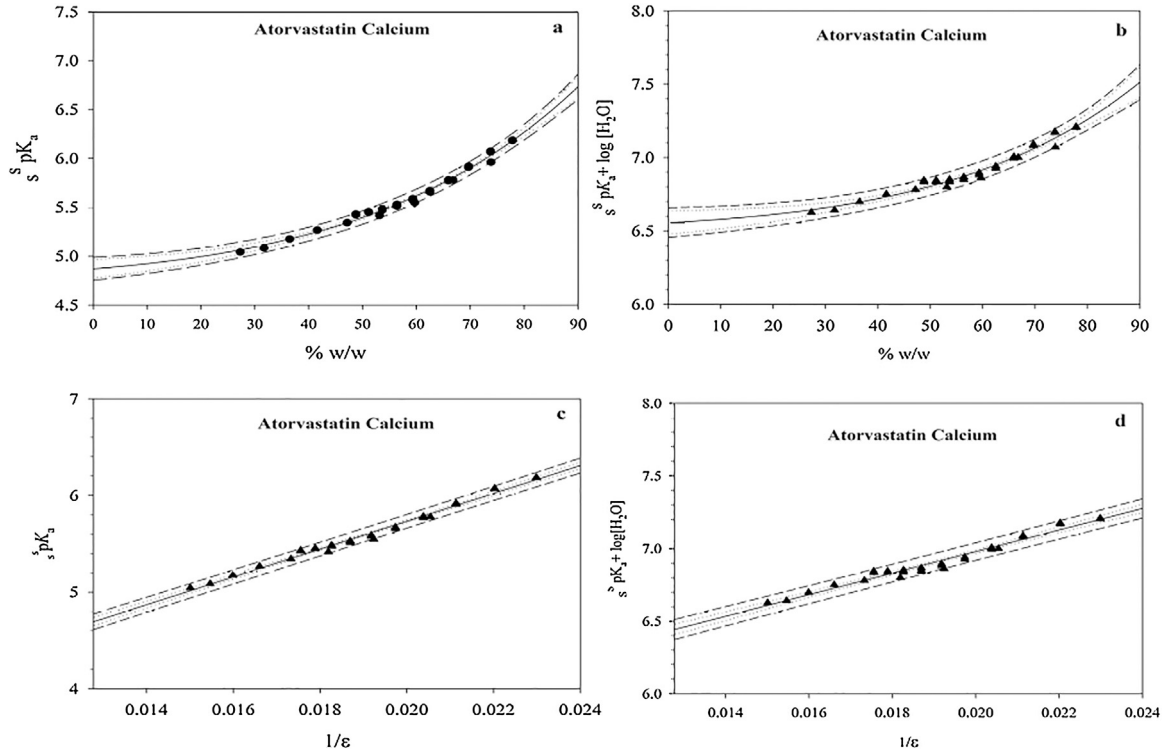

Fig. 3. Empirical regressions of ${}^{s}_{s}pK_a$ values for atorvastatin. Solid lines represent the regressions given as: Plot (a) ${}^{s}_{s}pK_a$ vs.%w/w; Plot (b) (${}^{s}_{s}pK_a$+log[H_2O]) vs.%w/w; Plot (c) ${}^{s}_{s}pK_a$vs. 1/ε; Plot (d) Yasuda-Shedlovsky: (${}^{s}_{s}pK_a$+ log[H_2O]) vs. 1/ε. Segmented line represent the 95% prediction bands, and dotted lines the confidence intervals.

butol. Atorvastatin is used primarily for prevention of events associated with cardiovascular disease, whereas the action of ethambutol is bactericidal that specifically inhibits the transfer of mycolic acids into the cell of the tubercle bacillus.

Graphical results of experimental data are shown in Fig. 3 for atorvastatin and in Fig. 4 for ethambutol. These Figures include plots with the three dependencies given by Eqs. (7), (8) or (9), in plots a, b and c, respectively, and dependence of Y-S model given by Eq. (6) in plot d.

The parameters of the regressions and calculated ${}^{w}_{w}pK_a$for these two compounds are summarized in Table 3. The correlation coefficients, r^2, of the empirical equation ranged between 0.948 and 0.990, which is acceptable for both compounds. Lower values of r^2 for ethambutol ${}^{s}_{s}pK_{a1}$ and ${}^{s}_{s}pK_{a2}$are attributed to the relatively poor dependencies between dissociation constants and changes in solvent composition (very small slopes in their regression). To evaluate the accuracy of the proposed equation, plot of residuals of were made and plotted against solvent composition. Residuals were distributed homogeneously between ±0.02 logarithmic units (data not shown).

4. Conclusions

We have proposed a fast and fully automated method (FSM) for the accurate determination of thermodynamic dissociation constants of pharmaceutical drugs in different solvent/water mixtures

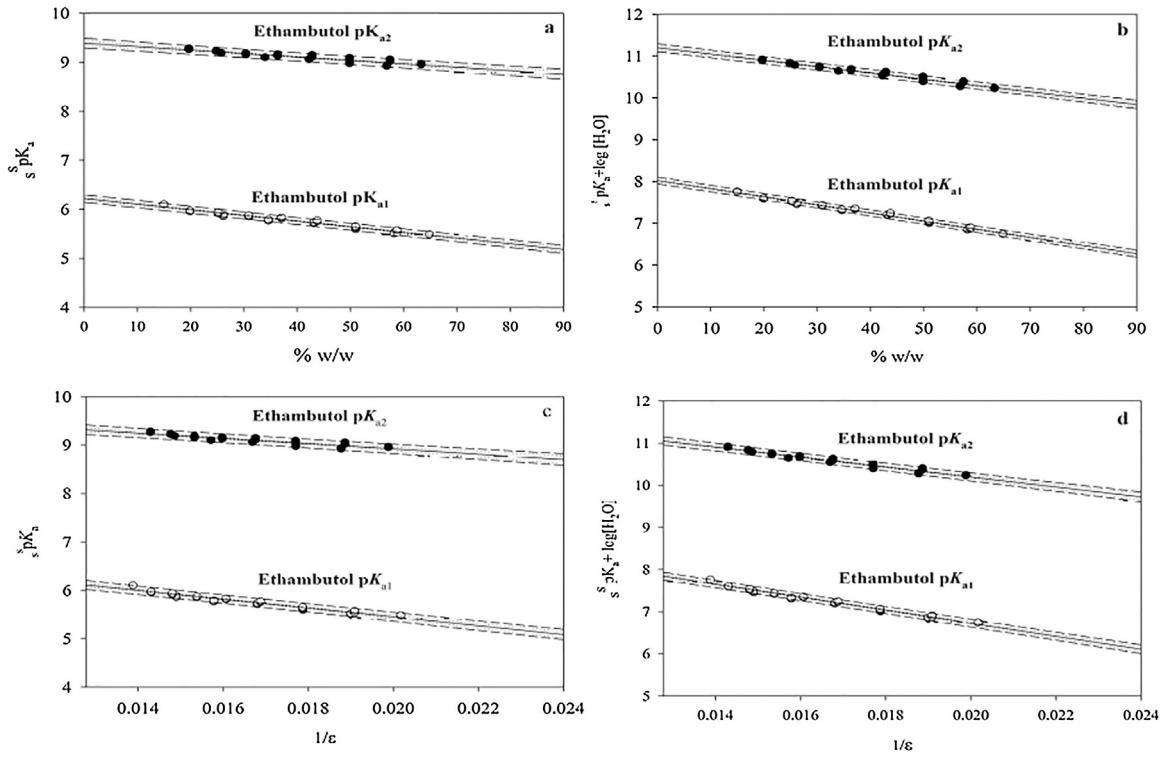

Fig. 4. Empirical regressions of ${}^{s}_{s}pK_a$ values for Ethambutol ${}^{s}_{s}pK_{a1}$ (○) and for Ethambutol ${}^{s}_{s}pK_{a2}$ (●). Solid lines represent the regressions given as: Plot (a) ${}^{s}_{s}pK_a$ vs.%w/w; Plot (b) $({}^{s}_{s}pK_a + \log[H_2O])$ vs.%w/w; Plot (c) ${}^{s}_{s}pK_a$ vs. $1/\varepsilon$; Plot (d) Yasuda-Shedlovsky: $({}^{s}_{s}pK_a + \log[H_2O])$ vs. $1/\varepsilon$; Segmented line represent the 95% prediction bands, and dotted lines the confidence intervals.

Table 3
Parameters and Results obtained from fits of experimental ${}^{s}_{s}pK_a$ to solvent mixture composition properties for atorvastatin calcium and ethambutol (first and second ionization constants) at 25 °C. S. D. of each value indicated in parentheses.

Compound	Regression	y_o	a	b	${}^{w}_{w}pK_a$	$r^{2\,d}$
Atorvastatin	${}^{s}_{s}pK_a$ *vs.* %w/w[a]	4.87 (0.05)	4×10^{-3} (9×10^{-3})	17×10^{-3} (2×10^{-3})	4.87 (0.05)	0.99
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs* %w/w[a]	6.56 (0.04)	2×10^{-3} (0.7×10^{-3})	19×10^{-3} (4×10^{-3})	4.82 (0.04)	0.97
	${}^{s}_{s}pK_a$ *vs* $1/\varepsilon$[b]	2.85 (0.06)	144 (3)	–	4.76 (0.05)	0.99
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs* $1/\varepsilon$[c]	5.50 (0.05)	74 (3)	–	4.70 (0.04)	0.97
Ethambutol (pK_{a1})	${}^{s}_{s}pK_a$ *vs* %w/w[b]	6.22 (0.02)	-12×10^{-3} (0.4×10^{-3})	–	6.22 (0.04)	0.97
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs* %w/w[b]	8.03 (0.02)	-19×10^{-3} (0.4×10^{-3})	–	6.28 (0.04)	0.99
	${}^{s}_{s}pK_a$ *vs* $1/\varepsilon$[b]	7.28 (0.07)	−91 (4)	–	6.11 (0.05)	0.95
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs.* $1/\varepsilon$[c]	9.82 (0.07)	−154 (4)	–	6.09 (0.05)	0.98
Ethambutol (pK_{a2})	${}^{s}_{s}pK_a$ *vs* %w/w[b]	9.39 (0.03)	-7.0×10^{-3} (0.6×10^{-3})	–	9.39 (0.05)	0.85
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs* %w/w[b]	11.20 (0.02)	-15×10^{-3} (0.6×10^{-3})	–	9.46 (0.05)	0.97
	${}^{s}_{s}pK_a$ *vs* $1/\varepsilon$[b]	10.01 (0.08)	−55 (5)	–	9.32 (0.06)	0.83
	${}^{s}_{s}pK_a + \log[H_2O]$ *vs.* $1/\varepsilon$[c]	12.56 (0.08)	−118 (5)	–	9.31 (0.05)	0.96

[a] Data fitted to the empirical equation: $f = y_0 + a.xy_0 + a{\cdot}x\ \exp(bx)$.
[b] Data fitted to $f = y_0 + a.x$.
[c] Data fitted to Yasuda-Shedlovsky expression.
[d] r^2 denotes the correlation factors.

where the solubility is significantly, aiming to extrapolate the value to the corresponding to pure water, where the low solubility can make difficult its determination. Based on two solutions of the target compound and an automated instrument, a large data set of thermodynamic ${}^{s}_{w}pH$ values at different solvent/water compositions can be obtained from two single sequences of additions in less than two hours. These set of values can be, firstly, converted into ${}^{s}_{s}pH$ and, then, in thermodynamic ${}^{s}_{s}pK_a$ values. In order to validate the method and evaluate different possible extrapolations, using methanol/water mixtures the method was applied to a group of known pharmaceutical drugs taken as models of: carboxylic acids (salicylic acid and ibuprofen), phenols (atenolol) and amines (labetalol). For these compounds, thermodynamic dissociation constants given as ${}^{s}_{s}pK_a$ or $({}^{s}_{s}pK_a + \log[H_2O])$ were analyzed versus different solvent composition properties: reciprocal dielectric constant or percent by weight. Using 1/e as variable, the behaviors are very linear when dealing with dissociations of neutral molecules (carboxylic acids and phenols), although it strongly deviates for solvent contents higher than 65% by weight when the molecule to be dissociated is charged (protonated amine). Using $({}^{s}_{s}pK_a + \log[H_2O])$ and percent by weight, the behavior is not linear but is the only case where experimental data do not deviates from the trend for methanol contents above 65% by weight, which makes this simple combination the more promising. Extrapolated ${}^{w}_{w}pK_a$ values are compared with values measured obtained with several techniques by different authors observing good agreement. Finally, FSM is applied to determine ${}^{s}_{s}pK_a$ of ethambutol and atorvastatin calcium along a wide range of methanol composition, and using four expressions, the dependencies of ${}^{s}_{s}pK_a$ on methanol content are correlated and ${}^{w}_{w}pK_a$ in pure water is calculated. The FSM combined

with extrapolation method resulted a fast, experimentally easy, accurate and promising method to characterize drug candidates soluble or with low solubilities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jpba.2018.03.010.

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