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Determination of the pK_a values of β-blockers by automated potentiometric titrations

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Abstract

The acid–base equilibrium constants of the β-blockers atenolol, oxprenolol, timolol and labetalol were determined by automated potentiometric titrations. The pK_a values were obtained in water-rich or water–methanol medium (20% MeOH) to obviate the solubility problems associated with the compounds. The initial estimates of pK_a values were obtained from Gran's method and then, were refined by the NYTIT and ZETA versions of the LETAGROP computer program. The resultant values were 9.4 ($I = 0.1$ M KCl, 20% methanol) for atenolol, 9.6 ($I = 0.1$ M KCl) for oxprenolol, 9.4 ($I = 0.1$ M KCl, 20% methanol) for timolol and 7.4 and 9.4 ($I = 0.1$ M KCl) for labetalol. The potentiometric method was found to be accurate and easily applicable. The operational criteria for applying the methodology are indicated.

Keywords: Dissociation constants; Potentiometric titrations; β-Blockers

1. Introduction

β-Adrenergic blocking drugs are of therapeutic value in the treatment of various cardiovascular disorders, such as angina pectoris, cardiac arrhythmia and hypertension. A great number of β-blockers are available, which differ not only in their specific β-adrenoceptor blocking effects, but also in their non-specific effects [1,2].

These substances are subjected to restrictions in sport. Their abuse was forbidden in 1987 by the International Olympic Committee (IOC) in sports such as shooting, pentathlon, ski-jumping and billiards [3].

β-Adrenergic blocking agents, as a class of drugs, have one common structural feature, either an ethanolamine (labetalol) or an oxypropanolamine (atenolol, oxprenolol and timolol) side chain, which possess a secondary amine group. At physiological pH value, the amine group is singly charged, and the compound shows the largest pharmacological activity.

In addition to their pharmacological interest, equilibrium data are of importance in describing and understanding the mechanism of action of the β-blockers. Further, the effectiveness of the drugs depends on their route of administration. The pK_a value of the drug can be decisive in determining

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the passage of the drug across membranes within the body adsorption, tissue distribution and elimination. Thus, the knowledge of the acidity constants is also necessary to choose the best conditions for the extraction of the drugs from body fluids, and in order to develop chromatographic and electrophoretic methods that allow their determination.

Despite the importance of a precise knowledge of the dissociation constants of the β-blockers in pharmacological studies, the values reported in the literature show large variations and even in a review, some of the values quoted are wrongly assigned [4].

The pK_a values reported in literature have been determined by potentiometry in aqueous [4], acetonitrile media [5,6], methanol–water [7] and different isotropic biphasic solvent systems [8] or liquid–liquid extraction from the octanol–water partition data [1,9] and in some cases, the method applied is not clearly described [10].

Potentiometric titrimetry in aqueous solutions is the most precise method for the determination of equilibrium constants, once a model to describe adequately the system has been chosen. The equivalence point in potentiometric titrations is generally determined by plotting E or pH as a function of volume of the reagent added. The equivalence point is said to lie at the inflexion point of the curve. In the titration of very weak acids, the slope of the curve at this point can be so small that it is difficult to determine the exact equivalence point with any great degree of accuracy. In these cases, it is possible to obtain better results by using various graphical or numerical methods.

pK_a determination of water-insoluble drugs has been successfully carried out by means of Sirius potentiometric equipment [11,12].

Cazallas et al., reported an automated potentiometric titration system applying the POSPETR program developed in our department [13]. This system was applied to the study of the acid–base equilibrium of the benzodiazepine loprazolam [14].

The aim of the present study is to apply the automated potentiometric system indicated above to the determination of the dissociation constants of four β-blockers. The compounds studied are atenolol (4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetamide), oxprenolol hydrochloride (1-(2-allyloxyphenoxy)-3-isopropylamino propan-2-ol hydrochloride), timolol maleate (−)-(S)(1)-*tert*-butylamino-3-(4-morpholinyl-1,2,5-thiadizol-3-yloxy) propan-2-ol hydrogen maleate), and labetalol hydrochloride (5-[(*RS*)-1-hydroxy-2-[(*RS*)-1-methyl-3-phenylpropyl)amino]ethyl] salicylamide hydrochloride).

2. Experimental

2.1. Apparatus

The experiments were carried out as potentiometric titrations with constant ionic strength using an automated system, applying the POSPETR program [13].

The titration system was composed of a titration cell with a magnetic stirrer. All titrations were performed under a nitrogen atmosphere to avoid CO_2 contamination and the temperature was controlled at 25 ± 0.1°C. A cell with the following general composition was used to measure the $[H^+]$ concentration.

Ag, AgCl (s) $|I$ mol dm^{-3} KCl saturated in AgCl B% methanol$\|$Test solution$|$Glass electrode

where I is the ionic strength. The glass electrode and the reference electrode used were Metrohm 6.0101.000P.E. and Metrohm 6.0726.100R.C., respectively.

All titrations were performed by stepwise additions of the titrant with an automatic burette Metrohm Dosimat 725. The system was controlled by a PC/AT microcomputer via a Hewlett Packard 3421A data acquisition and control unit.

A Shimadzu UV-260 double beam spectrophotometer was used for absorbance measurements (1 cm of length path).

2.2. Reagents and solutions

All β-blockers were supplied by Sigma (Barcelona, Spain). Methanol was Lab-Scan HPLC grade (Bilbao, Spain). All reagents were Merck Suprapur (Barcelona, Spain). Water was

obtained from Milli-RO and Milli-Q Water systems.

Stock solutions of the β-blockers (1000 μg ml^{-1}) were prepared in water (oxprenolol hydrochloride and labetalol hydrochloride) or methanol (atenolol and timolol maleate), and were kept in dark and stored under refrigeration at 4°C to minimise decomposition.

Stock solutions of 1 M hydrochloric acid and 1 M potassium hydroxide were also prepared. These solutions were standardised volumetrically against mercury(II) oxide and potassium hydrogen phthalate, respectively.

3. Results and discussion

The potentiometric technique requires the selection of an ionic medium with constant strength in order to ensure that the activity coefficients remain constant for all the species within the experiments.

The major difficulty in obtaining reliable values for the dissociation constants of β-blockers by potentiometry is due to their low solubility in aqueous solutions. A high concentration of the drug is required ($\geq 10^{-4}$ M) in order to obtain a worthy pH jump at the equivalence point. On the other hand, some β-blockers are sparingly soluble in aqueous saline solutions, especially at basic pH values. So, an hydroalcoholic medium is used to enhance their solubility.

3.1. Determination of the pH

In the titration method, free hydrogen ion concentration, $[H^+] \equiv h$, was determined by measuring the e.m.f. of the cell (E). At 25°C, the e.m.f. can be calculated by the Nernst equation [15].

$$E = E'_o + E_j(h) + 59.16 \log h$$

where E'_o is the formal potential of the cell at a given ionic strength. The liquid junction potential term, E_j may be expressed as [16].

$$E_j(h) = j_{ac}h + j_{alk}K_w h^{-1}$$

where j_{ac} and j_{alk} are the liquid junction constants in the acid and the alkaline regions, respectively, and K_w is the stoichiometric autoprotolysis constant of water in the saline medium at a given methanol percentage.

E'_o, j_{ac} and j_{alk} are constant during each titration, but it is well known that the formal potential of the cell, E'_o, varies from day to day [17] and so it is difficult to reproduce liquid-junction potentials with adequate precision [18]. For this reason, ionic medium titrations were performed before each titration.

The titrations of these saline solutions were conducted in a medium with the same composition (ionic strength and methanol percentage) as that where the pK_a determinations of the β-blockers were carried out. The reference electrode was filled with an internal solution that contained the same methanol percentage as the titrated solutions, and it was left to stabilise during 24 h before the titration.

Hence, an aliquot (50 ml) of ionic medium solution containing 0.01 M HCl in water or water–methanol was titrated with KOH (C_1) and another 50 ml aliquot of the saline solution was titrated with a strong acid (HCl) (C_2). The titrant solutions were prepared as following:

$$\begin{aligned} C_1 &= A_1 \text{ mol dm}^{-3} \text{ KOH} \\ &\quad + (I - A_1) \text{ mol dm}^{-3} \text{ KCl} \\ &\quad + B\% \text{ v/v methanol} \\ C_2 &= A_2 \text{ mol dm}^{-3} \text{ HCl} \\ &\quad + (I - A_2) \text{ mol dm}^{-3} \text{ KCl} \\ &\quad + B\% \text{ v/v methanol} \end{aligned}$$

where I is the ionic strength 0.1 or 0.5 M, A_1 and A_2 are 0.1 or 0.2 M, and B can be 0 or 20%. In the titrations at 0.1 M ionic strength, the concentration of the titrant was 0.1 M and so it was not necessary to add KCl. But in the titrations at 0.5 M ionic strength, the concentration of the titrant was 0.2 M and KCl was added to reach the I value.

3.2. Titrations of the β-blockers

The acidity constants of oxprenolol and labetalol were obtained in water at constant ionic

strength (0.1 and 0.5 M KCl), whereas the determination of the pK_a of timolol and atenolol were performed in a hydroalcoholic solution (20% methanol) at constant ionic strength of 0.1 M KCl.

These solutions (50 ml) were pre-acidified to approximately pH 3 with 1 M HCl, and were then titrated alkalimetrically until pH 12 with 0.1 M KOH (titrant solution C_1). The test solutions were prepared with the following general composition.

$C_B = C$ mol dm^{-3} β-blocker
$+ D$ mol dm^{-3} HCl
$+ (I - C - D)$ mol dm^{-3} KCl
$+ B\%$ v/v MeOH

where I is the ionic strength, C is the concentration of the β-blocker and D is the concentration of the acid in excess (Table 1).

The titration curve obtained for atenolol and oxprenolol shows two potential jumps (Fig. 1). The first one can be assigned to the hydrochloric acid in excess and the second one to the β-blocker. The titration curve of timolol shows three potential jumps. The first one corresponds to the hydrochloric acid in excess, the second one to the maleic acid and the last one to the drug. In the case of the titration of labetalol, three jumps are also detected. The first one is assigned to the deprotonation of the acid in excess and the other two to the compound. In all the titrations, the potential jump obtained for the hydrochloric acid is better defined than for the β-blockers because these are weak acids.

3.3. Treatment of the potentiometric data

The experimental data in the potentiometric titrations (E, V) were transformed into (Z, $\log h$) data. Z is the average number of ligands (H^+) which are bound to the compound. For each experimental point, h can be calculated from the Nernst equation using the Newton–Raphson iterative procedure and the average number of protons bound can be calculated from:

$$Z = \frac{[H^+]_{Tot} - [H^+] + [OH^-]}{[\beta\text{-blocker}]_{Tot}}$$

$$[H^+]_{Tot} = \frac{C_{HCl}V_0 + nCV_0 - [OH^-]V_T}{V_0 + V_T}$$

$$[OH^-] = K_w h^{-1}$$

$$[\beta\text{-blocker}]_{Tot} = \frac{CV_0}{V_0 + V_T}$$

provided that C, the total concentration of β-blocker; $[OH^-]_T$, the total hydroxyl concentration in the titrant; $C_{HCl} = D$, the HCl concentration at the beginning of the titration; V_0, the initial volume and V_T, the titrant volume added, are known and where, $n = 1$ or 2 depending on the number of H^+ which take part in the acid–base equilibrium.

The formation curves of atenolol and oxprenolol (Fig. 2) show that one hydrogen ion is bound to the compound, so only one pK_a value exists. On the other hand, for timolol and labetalol, two pK_a values can be defined ($Z = 2$) although the plateau at $Z = 1$ is not clearly defined due to the nearness of the pK_a values.

Table 1
Titration conditions for the test solutions of the β-blockers

	Atenolol	Timolol	Oxprenolol		Labetalol	
C (mol dm^{-3})	4×10^{-3}	3×10^{-3}	1×10^{-2}	1×10^{-2}	3×10^{-3}	3×10^{-3}
D (mol dm^{-3})	6×10^{-3}	4×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
I (mol dm^{-3})	0.1	0.1	0.1	0.5	0.1	0.5
$B\%$ (v/v)	20	20	0	0	0	0

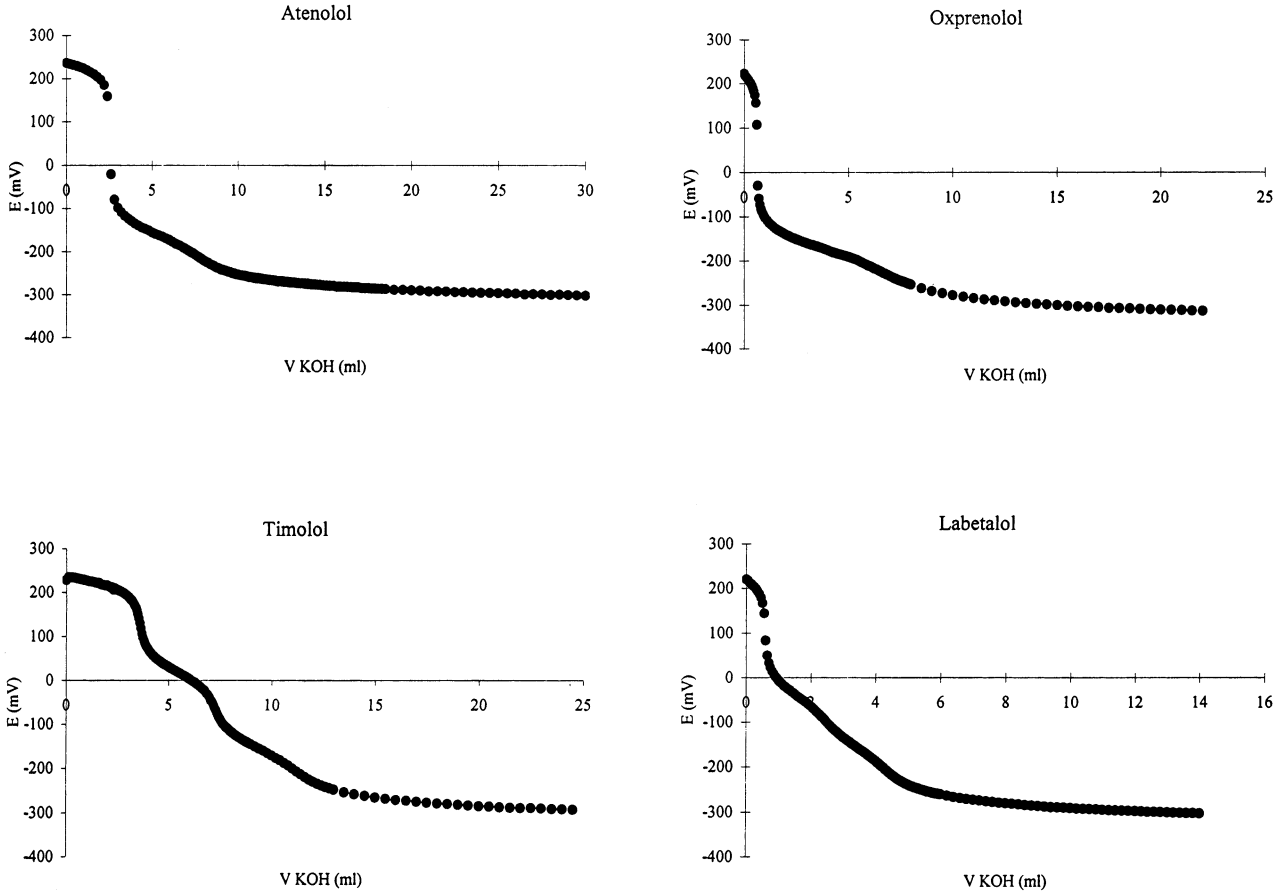

Fig. 1. Potentiometric titration curves for the β-blockers studied. Atenolol, 4×10^{-3} M, $I = 0.1$ M KCl, 20% methanol; oxprenolol, 1×10^{-2} M, $I = 0.5$ M KCl; timolol, 3×10^{-3} M, $I = 0.1$ M KCl, 20% methanol, and labetalol, 3×10^{-3} M, $I = 0.1$ M KCl.

The potentiometric data obtained were treated graphically as well as numerically. The acid–base equilibrium of the compounds oxprenolol and atenolol can be represented by:

$$H^+ + R \rightleftarrows RH^+$$

with the corresponding stoichiometric constant:

$$\beta = \frac{[RH^+]}{h[R]} \quad (\beta = K^{-1})$$

The graphical treatment was performed using a normalised variable method. Defining the normalised variable $X = \beta h$ and taking into account the mass balance equations for the β-blockers, the average number of ligands, Z, can be obtained by:

$$Z = \frac{\beta h}{1 + \beta h} = \frac{X}{1 + X}$$

The difference on the x-axis between the experimental (Z vs. $\log h$) and the theoretical functions (Z vs. $\log X$) in the position of best fit allows the determination of the stoichiometric constant according to:

$$\log X = \log \beta - \log h$$

In the case of labetalol and timolol the acid–base equilibrium can be expressed by:

$$R^- + H^+ \rightleftarrows RH \quad \beta_1$$

$$R^- + 2H^+ \rightleftarrows RH_2^+ \quad \beta_2$$

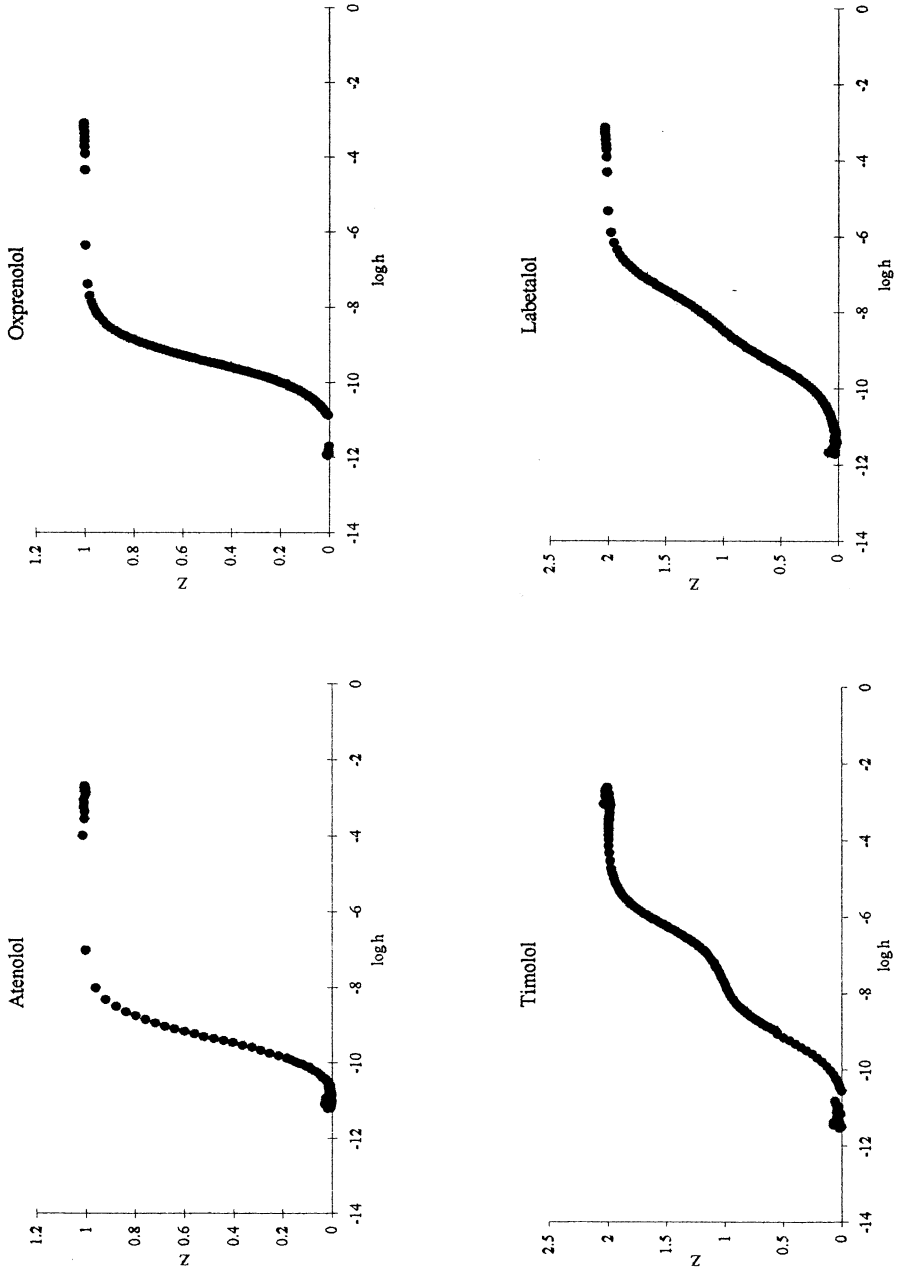

Fig. 2. Formation curves of the β-blockers.

$$\beta_1 = \frac{[RH]}{h[R^-]} \quad \beta_1 = (Ka_2)^{-1}$$

$$\beta_2 = \frac{[RH_2^+]}{h^2[R^-]} \quad \beta_2 = (Ka_1 \times Ka_2)^{-1}$$

The graphical treatment was performed using a normalised variable and a parameter method. Defining the normalised variable $X = (\beta_2)^{1/2}h$, the parameter $p = (\beta_1/\beta_2)^{1/2}$, and taking into account the mass balance equations for the β-blockers, the following equation is obtained:

$$Z = \frac{(\beta_1 h + 2\beta_2 h^2)}{(1 + \beta_1 h + \beta_2 h^2)} = \frac{(pX + 2X^2)}{(1 + pX + X^2)}$$

The difference on the x-axis between the experimental (Z vs. $\log h$) and the theoretical functions (Z vs $\log X$), once the best p value has been selected, in the position of best fit allows the determination of the stoichiometric constant according to:

$$\log X = \frac{1}{2}\log \beta_2 - \log h$$

The numerical treatment was carried out with the NYTIT and ZETA versions of the LETAGROP program [19], which treats the original (E, V) or (Z, V) data respectively, minimising the sum of the squared deviations, U_E or U_Z, for all Np experimental points:

$$U_E = \sum_{Np}(E_{cal} - E_{exp})^2$$

$$U_E = \sum_{Np}(Z_{cal} - Z_{exp})^2$$

where E_{exp} and E_{cal} or Z_{exp} and Z_{cal} are the experimental and calculated values, respectively. The NYTIT program defines j_{ac}, j_{alk} and K_w as common parameters for all the titrations and the E_o value as a refinable parameter for each titration. However, the ZETA program does consider neither E_j nor E_o, but it allows detecting and correcting systematic errors. The initial estimates pK_a values obtained by Gran's method [20] are refined by these numerical methods. The pK_a values calculated by graphical and numerical methods at different ionic strengths are collected in Table 2.

An unique pK_a value for atenolol, timolol and oxprenolol has been calculated. These compounds have similar pK_a values (around 9.4). This means that at physiological pH (7.4) they exist as singly-charged cations due to protonation of the amine functional group (Fig. 3).

The potentiometric titration of timolol maleate allows the calculation of two acidity constants for this compound. The potentiometric data obtained for this β-blocker have been treated, as if, it was a system with two dissociation constants. Only the second pK_a (9.4) value can be assigned to the dissociation of the amine functional group. The other pK_a value obtained (6.22) corresponds to the deprotonation of the maleic acid, which is in agreement with the tabulated for this acid. Laxer

Table 2
pK_a values obtained by different potentiometric methods for the β-blockers under study

β-Blocker	Graphical methods		Numerical methods	
	Gran	Normalisation	ZETA	NYTIT
Atenolol ($I = 0.1$ M)	9.40	9.32 ± 0.05	9.28 ± 0.03	9.39 ± 0.05
Timolol ($I = 0.1$ M)	9.34	9.34 ± 0.25	9.11 ± 0.02	9.38 ± 0.13
Oxprenolol ($I = 0.1$ M)	9.56	9.50 ± 0.05	9.58 ± 0.08	9.58 ± 0.01
Oxprenolol ($I = 0.5$ M)	9.62	9.60 ± 0.05	9.41 ± 0.01	9.45 ± 0.01
Labetalol ($I = 0.1$ M)	7.35	7.38 ± 0.24	7.38 ± 0.02	7.37 ± 0.05
	9.42	9.36 ± 0.22	9.37 ± 0.01	9.41 ± 0.03
Labetalol ($I = 0.5$ M)	7.44	7.47 ± 0.24	7.45 ± 0.02	7.45 ± 0.04
	9.52	9.43 ± 0.22	9.37 ± 0.02	9.42 ± 0.02

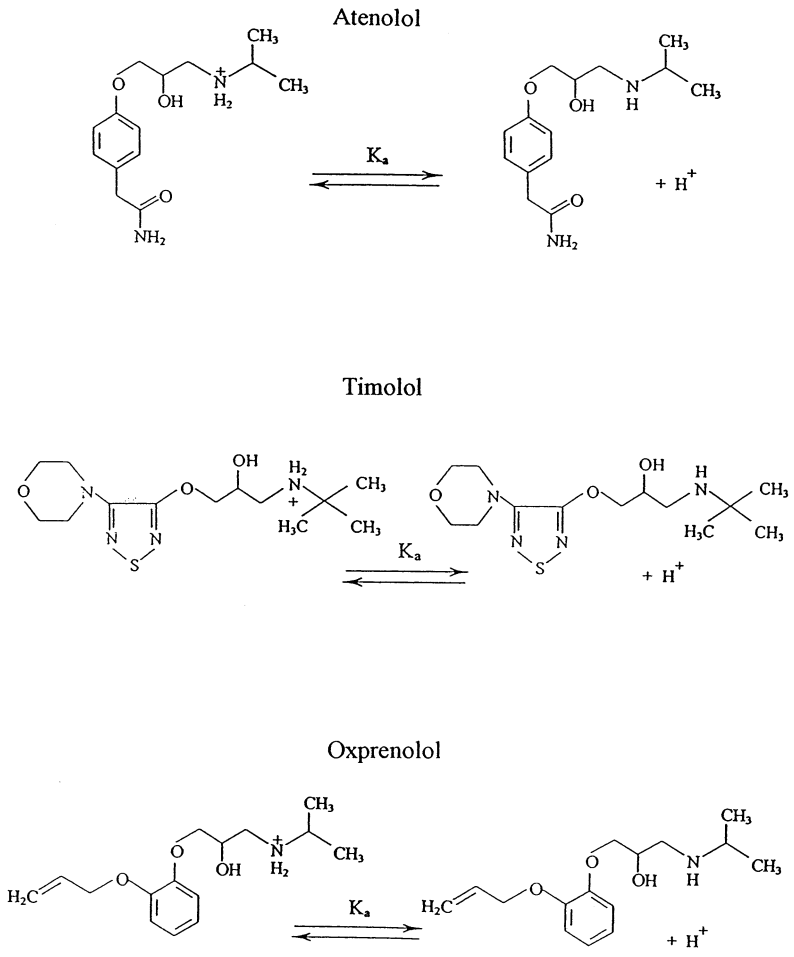

Fig. 3. Acid–base equilibrium of the β-blockers atenolol, timolol and oxprenolol.

et al. proposed two pK_a values for timolol because they did not consider the maleic acid equilibrium [4]. The first pK_a value for the maleic acid (1.92) can not be calculated in the experimental conditions of the potentiometric titrations carried out.

It is worthy noting that two acidity constants have been determined for labetalol, which

Table 3
pK_a values of the β-blocker labetalol obtained by UV-spectrophotometry

Graphical methods				Numerical methods
Normalisation		Projection strip		LETAGROP-SPEFO
7.26[a]	9.26[a]	7.20[a]	9.28[a]	7.60 ± 0.21
7.44[b]	9.26[b]	7.32[b]	9.32[b]	9.42 ± 0.10

[a] $\lambda = 300$ nm.
[b] $\lambda = 332$ nm.

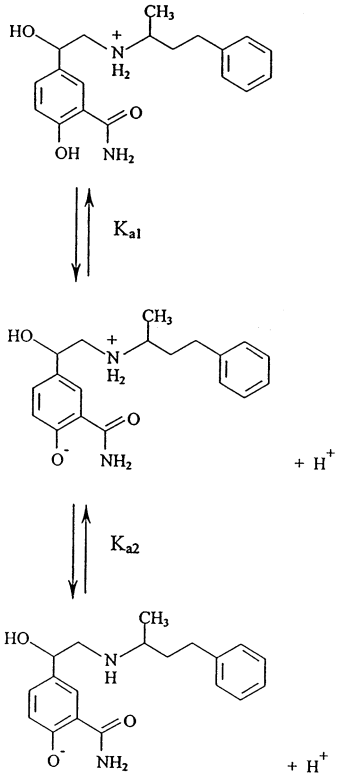

Fig. 4. Acid–base equilibrium of the β-blocker labetalol.

strengthen its amphiprotic behaviour. The first pK_a value (7.4) can be attributed to the ionisation of the phenolic group (Fig. 4), taking into account that this group has more acid character than the amine group [21]. This singularity makes the compound an unique β-blocker under study which acts simultaneously on β- and α-adrenoceptors [1]. This value (7.4) is in accordance with that reported by Barbato et al. [9] although, V. Marko proposed only one pK_a value for this compound [10].

The pK_a values of labetalol were confirmed spectrophotometrically by graphical (normalisation and projection strip) [22] and numerical (LETAGROP-SPEFO) [19] methods, as can be seen in Table 3. The spectrophotometric technique was not applicable to the other β-blockers studied since the variation of the absorption spectra with pH was negligible.

4. Conclusions

Automated potentiometric titrations are reliable, accurate and easily applicable and they are a suitable method for the determination of the dissociation constants of the β-adrenoceptor blocking agents.

An important advantage of the potentiometric titration technique is that it can be applied to the determination of the pK_a values of compounds such as atenolol, oxprenolol and timolol, which can not be obtained by spectrophotometric methods.

On the other hand, it is worthwhile to point out that the automation of potentiometric titrations allows achieving a large number of data which can improve the definition of the titration curves and so increase the accuracy of the pK_a values collected.

The values obtained for all the compounds applying the different methods for the treatment of the potentiometric data are all in agreement.

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