Electrochemical Characterization of Tacrine, an Antialzheimer's Disease Drug, and Its Determination in Pharmaceuticals

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Abstract

Tacrine, 9-amino-1,2,3,4-tetrahydroacridine, an antialzheimer's disease agent was electrochemically studied and a polarographic method for its quantitative determination in pharmaceuticals was developed. Tacrine was electrochemically reduced and oxidized in aqueous medium, but for analytical purposes the cathodic behavior was prefered. According to the linear relation between the peak current and the tacrine concentration a differential pulse polarographic method was developed. Precision and accuracy of the developed method was checked by recovery studies. The proposed method was applied to the individual capsule assay (ICA) of the commercial capsules, showing that the uniformity content requirements were fulfilled. Alternatively a UV spectroscopic characterization was conducted, from the pH dependence of the absorbance bands an apparent pK_a of 10.44 was obtained. For comparative purposes a spectrophotometric determination also was developed.

Keywords: Tacrine, Differential pulse polarography, UV-spectrophotometry, Capsule assay

1. Introduction

Tacrine (THA, 9-amino-1,2,3,4-tetrahydroacridine) (Fig. 1). has potent anticholinesterase activity [1]. It has been used for the treatment of central anticholinergic syndrome caused by drug overdose [2, 3], intractable pain from terminal carcinoma [4], myasthenia gravis [5], and as a decurarizing agent [6]. Renewed interest in THA has been generated by its efficacy as therapy for memory loss [7], being the first drug approved by the U.S. Food and Drug Administration for the treatment of patients with Alzheimer's disease. Disposition studies in man indicate that THA undergoes extensive oxidative metabolism with a variety of mono and dihydroxylated metabolites excreted in urine [8].

Several articles describe high-perfomance liquid chromatographic (HPLC) methods for the analysis of plasma concentrations of THA alone, using either ultraviolet [9, 10] or fluorescence detection [11, 12]. Other articles describe the HPLC determination of tacrine and its metabolites in human plasma and urine using fluorescence or ultraviolet detection [12–14]. Also, electrochemical detection using a dual-electrode thin-layer cell has been used in the study of the metabolism of tacrine by rat hepatic microsomes [15].

In spite of the great number of published determinations of tacrine in biological fluids, there is only one article devoted to the determination of tacrine in pharmaceuticals [16]. This article is devoted to a spectrofluorometric determination of tacrine in pharmaceuticals and involve the separation of the excipients before the determination. Considering that the analysis of drug formulations, at the quality control level, in pharmaceutical laboratories or at the counter control (only take of sample) at the pharmacy office level are a growing need, new methods for pharmaceutical forms applications are required.

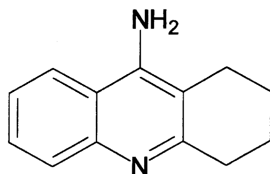


Fig. 1. Chemical structure of tacrine.

The electrochemistry of tacrine has been a totally untouched matter up to date. The only, rather tangential, reference related to the electrochemistry of tacrine is the tentative identification of a metabolite by comparison of its cyclic voltammograms with a standard [17].

The development of modern computer-bases electrochemical instrumentation, have turned the electrochemical methods very competitive from the analytical point of view. Furthermore, as generally excipients do not interfere in the polarographic determination, these methods present comparative advantages for the analysis of drug formulations.

This article reports the electrochemical behavior of tacrine and the development of a differential pulse polarographic procedure to determine its pharmaceutical dosage forms.

2. Experimental

2.1. Reagents and Solutions

Tacrine (100% chromatographically pure) and commercial capsules of Cognex (amount declared 30 mg tacrine per capsule) were obtained from Parke-Davis Laboratories (Santiago Chile). All the other reagents employed were of analytical grade.

Stock solutions of tacrine were prepared at a constant concentration of 0.025 M in distilled water. An aliquot of stock solution was taken and diluted in 0.1 M Britton-Robinson buffer adjusting the ionic strength at 0.3 M with KCl, to obtain a final working solution concentration between 5×10^{-6} and 1×10^{-3} M.

2.2. Apparatus

Electrochemical experiments, differential pulse polarography (DPP), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a totally automated Inelecsa assembly. A 25 mL thermostated Metrohm measuring cell, with three electrodes, dropping mercury electrode (DME), hanging mercury electrode (HME) and glassy carbon (GC) as a working electrode, a platinum wire counter electrode, and a saturated calomel reference electrode, were used for the measurements.

Spectrophotometric measurements were carried out with an UV-vis spectrophotometer ATI Unicam Model UV3, using 1 cm quartz cell and equipped with a 486 computer with vision acquisition and treatment program.

2.3. Analytical Studies

For calibration plots, a series of ten solutions contained tacrine concentrations between 2.5×10^{-5} and 1.5×10^{-4} M in Britton-Robinson buffer solution at pH 7.5 were prepared.

Excipients (cornstarch, magnesium stearate, lactose, sodium lauryl sulfate and talc) were added to the drug for recovery studies, according to manufacturer's batch formulas for 30 mg tacrine per capsule.

Capsule assay procedure was made with ten series of one capsule of Cognex (30 mg per capsule declared), which were suspended in water, sonicated and diluted to 100 mL. An aliquot (1 mL in DPP and 0.8 mL in UV-vis) of each solution was taken and diluted to 25 mL with Britton-Robinson buffer, pH 7.5.

In polarographic measurements, each sample was transferred to a polarographic cell, degassed with nitrogen for 5 min and recorded at least twice from −1500 to −1850 mV. For UV-vis measurements, each sample solution was centrifuged for 10 min at 4000 rpm. The obtained solutions were measured at 324 nm. The amount of tacrine in the sample solution was calculated from a prepared standard calibration plot.

For apparent pK_a (pK_a') determination, four bands (220, 240, 324 and 336 nm) were used. The pH solution was changed each 0.5 units. The temperature was kept constant at 25 °C. The tacrine concentration was 5×10^{-5} M for all the pH range.

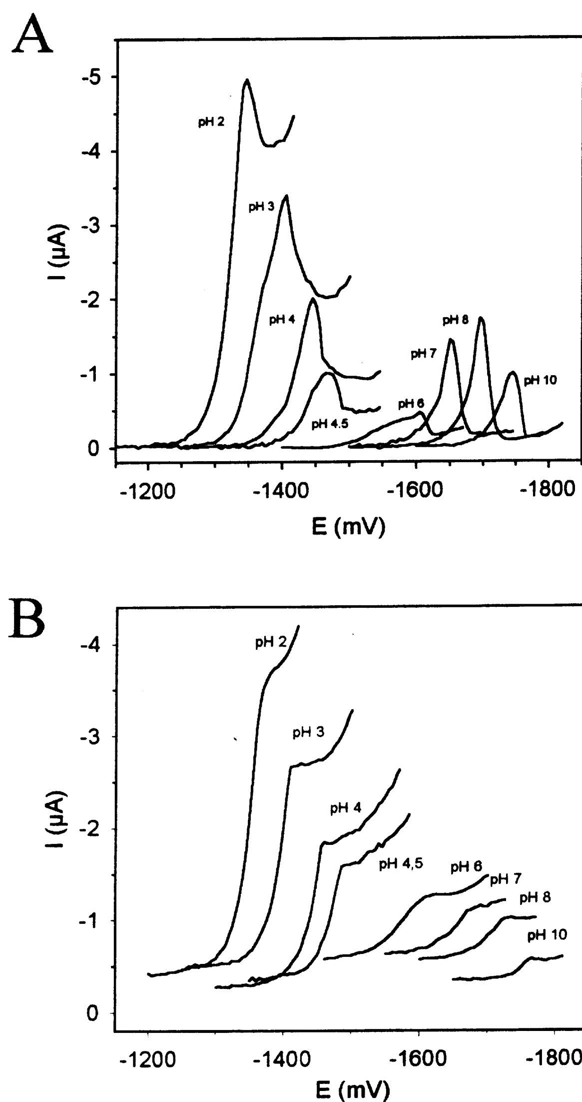


Fig. 2. Differential pulse (A) and tast (B) polarograms of 0.1 mM tacrine solution at different pH (0.1 M Britton-Robinson buffer, ionic strength 0.3 M KCl).

3. Results and Discussion

3.1. Cathodic Behavior

Tacrine was electrochemically reduced in a broad pH range using a dropping mercury electrode (DME), producing a rather complex signal at very high cathodic potential (Fig. 2).

From these polarograms it was possible to see that there is a change in the form (shape and height) of the peaks and waves (DPP and tast, respectively) when pH changes. Doing an exhaustively analysis at pH 6 (Fig. 3) it was clear that there are two peaks superimposed, indicating a change in the electroreduction mechanism of tacrine.

The evolution of peak potential with pH (Fig. 4) shows three linear segments with a discontinuity between pH 5.5–6. In the acidic and neutral range (pH < 8) the process was pH-dependent with slopes of 46.9 mV/pH between pH 2 and 5 and 39.1 mV/pH between pH 6 and 9.5. Up to pH 9.5 the peak potential was independent to the H^+ concentration.

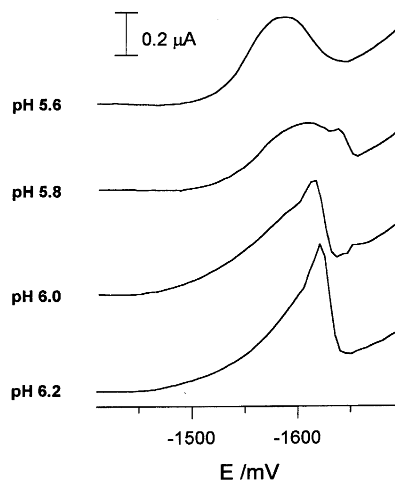


Fig. 3. Differential pulse polarograms of 0.1 mM tacrine solution between pH 5.6 and 6.2 (0.1 M Britton-Robinson buffer, ionic strength 0.3 M KCl).

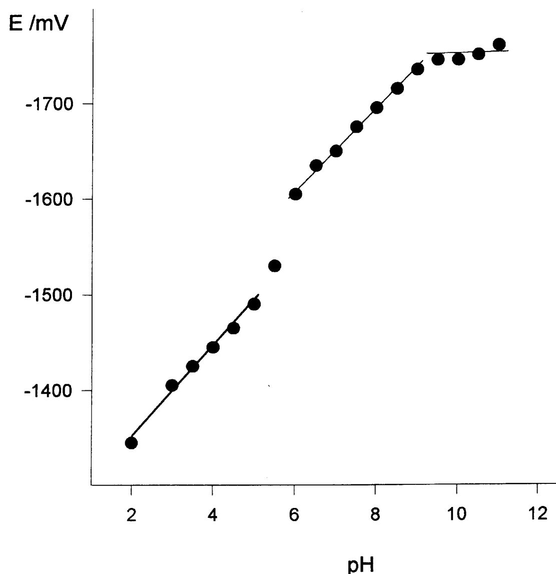


Fig. 4. pH dependence of the differential pulse potential peak of 0.1 mM tacrine solution.

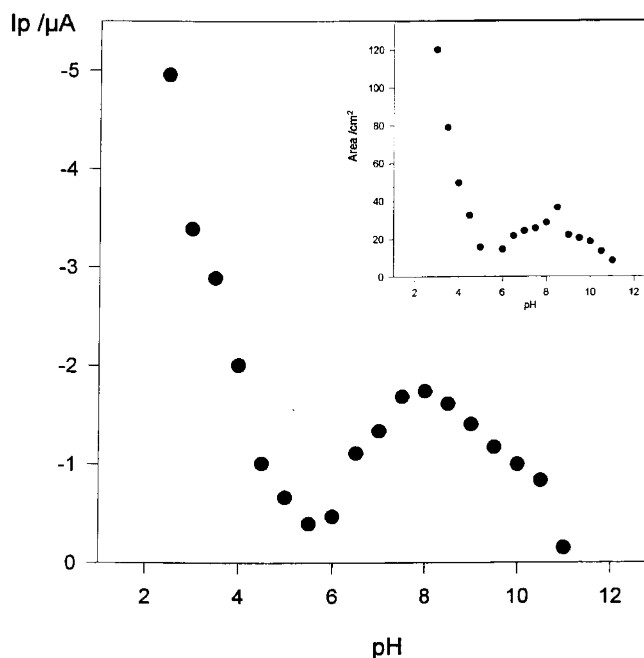


Fig. 5. pH dependence of the peak current and area (inset) of 0.1 mM tacrine solution.

Analyzing the evolution of the peak current (I_p) (Fig. 5), it is possible to observe that this parameter is affected by the pH value with a clear change at pH 5.5 and 8.5, in concordance with the breaks observed in the above E_p vs. pH plot. A similar response was observed in the peak area-pH plot, shown in the inset of Figure 5. However a totally different behavior was observed for the limiting current evolution with pH (Fig. 6), showing an exponential decay when pH increased. The above is a clear example of the different nature between peak current from DPP and limiting current from tast polarography. This is due to the fact that in DPP the height of the peak depends on the kinetics of the electrode process [18]. Limiting diffusion currents in tast polarography depend on the number of transferred electrons, in addition to the concentration of the analyte, the diffusion coefficient, and the size of the electrode, as given by the Ilkovic equation [19]. The peak current in DPP depends on all these factors, but additionally also on the rate constant of the electrode process and on the transfer coefficient. These aspects have also been noted by Zuman and Rupp [20] in recent work; we agree with their conclusion in the sense that, in order to obtain mechanistic information, limiting currents by DC or tast polarography are preferred. Consequently, we privilige the information obtained in Figure 6B in order to conclude about the pH-dependence of the limiting current. In this figure we can observe a strong decrease of the limiting current between pH 2–6. This behavior supports the postulation of a catalytic wave in this zone. Furthermore, in acidic media the limiting current reached a limiting value as the concentration was increased. Both of these behavior are typical of catalytical waves. However, between pH 7 and 8.5, the limiting current remains practically constant as a diffusion or adsorption wave.

Cyclic voltammetric experiments performed at pH 7.5 showed an adsorptive behavior of the electroreduction of tacrine (Fig. 7) with the appearance of an adsorptive post peak when tacrine concentration is increased. This post peak is due to strong adsorption of the reactant as described by the classical paper of Wopschall et al. [21]. This effect is also confirmed with the exponential dependence observed from the I_p vs. v^{12} plots at both concentrations (data not shown). From this experiment it was clear that the electrodic process was irreversible with or without adsorption of tacrine in the electrode.

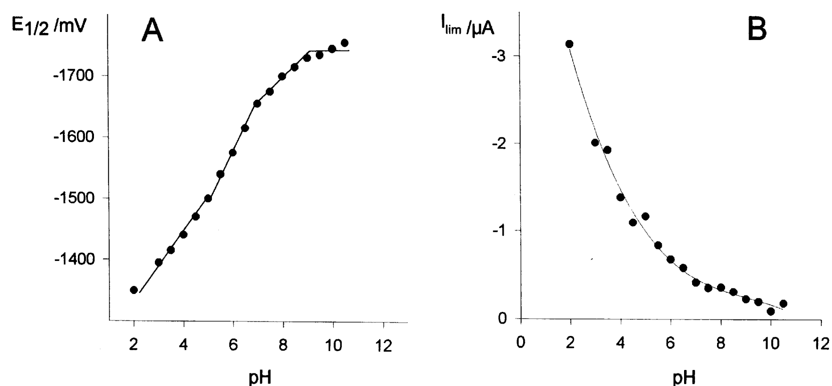


Fig. 6. pH dependence of the half-wave potential (A) and limiting current (B) of 0.1 mM tacrine solution.

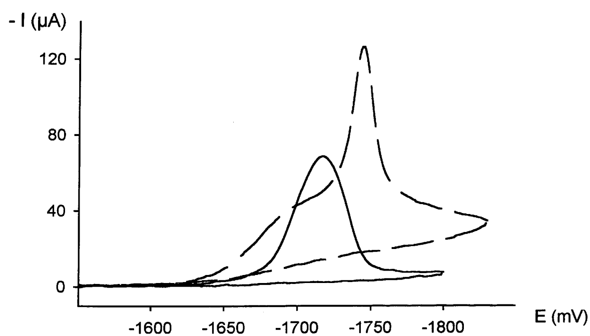
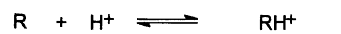
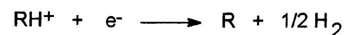


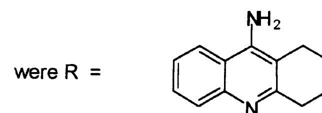
Fig. 7. Cyclic voltammograms of 0.1 mM (solid line) and 1 mM (dashed line) of tacrine solution at pH 7.5. Scan rate: 1 V/s.

The electrocapillary curves (Fig. 8), also show the occurrence of adsorption phenomena in the mercury electrode surface, and this effect is stronger in alkaline media (pH 8 and 10).

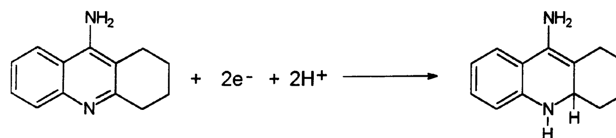
According to these results, we are able to postulate two different electroreduction processes for tacrine: 1) between pH 2 and ca. 6, tacrine produces a catalytic wave due to the proton reduction at lower potentials than the proper for this type of reduction:







and 2) in all the pH range the intracyclic azomethine group of tacrine is reduced according to:



Consequently, in acidic range the reduction of the azomathine group merges with the catalytic wave due to the proton reduction.

Fig. 8. Electrocapillary curves of tacrine at diffentent pHs.

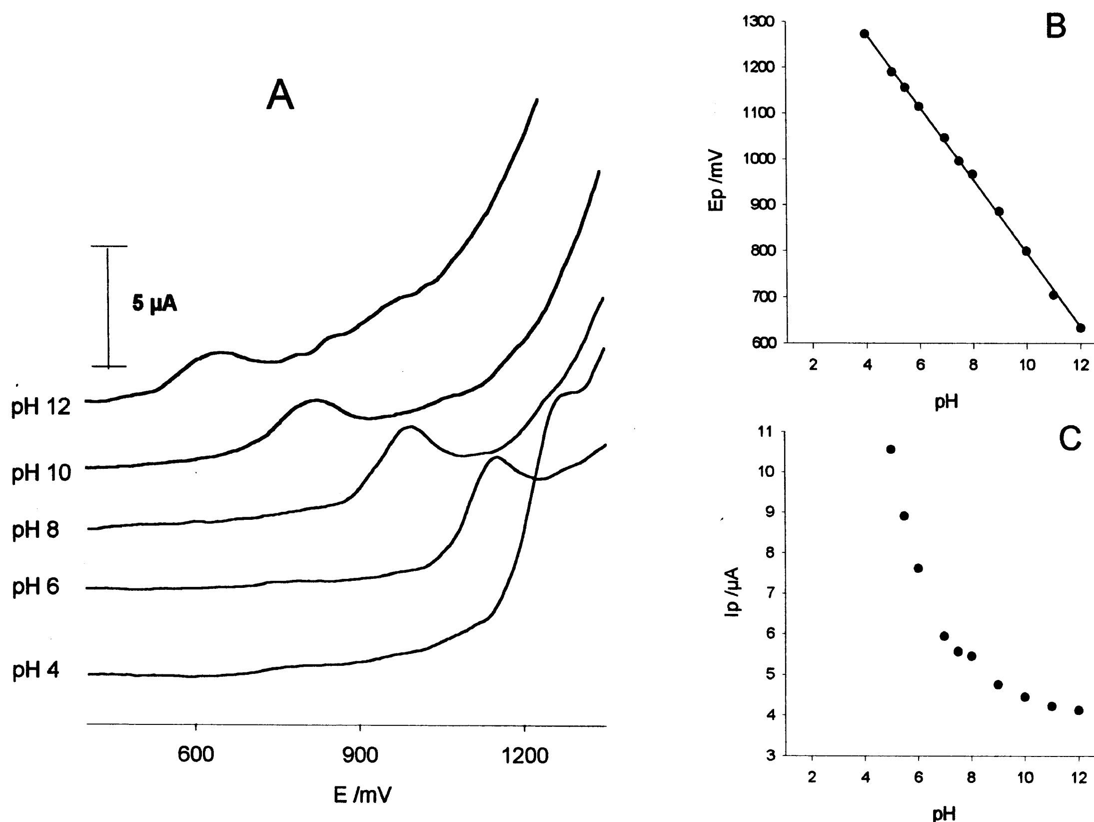


Fig. 9. Differential pulse voltammograms (A) of 0.1 mM tacrine solution at different pH and its peak potential (B) and peak current (C) dependence with pH.

3.2. Anodic Behavior

On the other hand, tacrine also was possible to be oxidizable on glassy carbon electrode between pH 4 and 12, producing a single signal by differential pulse voltammetry (DPV). In the acidic range (pH 4–5.5) this signal was poorly resolved and had the appearance as a shoulder of the solvent oxidation (Fig. 9A).

The E_p versus pH plot was linear in all the studied pH range (Fig. 9B). When the pH increase, the peak appeared at less anodic potentials and its intensity was lower (Fig. 9C). Due to the poorly resolved signal, further electrochemical studies were not conducted.

Table 1. Results of the recovery study of tacrine by DPP and UV-vis.

Assay [a]	*DPP*	*UV-vis*
Average recovery [%]	100.8	100.9
Standard deviation [%]	3.5	4.0
Coefficient of variation [%]	3.4	3.9

[a] Each average was calculated from ten independent samples.

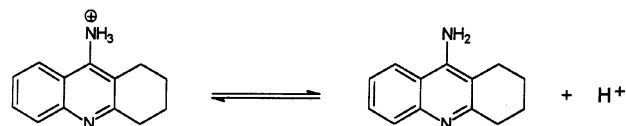
Table 2. Individual capsule assay results from comercial dossage forms of tacrine. Declared amount per capsule: 30 mg.

	DPP		*UV-vis*	
Assay	*mg/capsule*	*% found*	*mg/capsule*	*% found*
Average	29.10	97.0	29.54	98.5
s.d.	0.60	1.8	0.70	2.2
C.V. [%]	1.90	1.9	2.30	2.3

3.3. UV-Vis Spectrophotometric Characterization

With the aim of making a comparison between different techniques and to further explore the knowledge of the chemistry in solution of tacrine, a UV-visible spectrophotometric study was conducted.

UV-vis spectra at different pHs (Fig. 10A) reveal four absorption signals for tacrine, which are pH independent at the acidic and neutral range (pH 2–8) and pH-dependent above pH 8. The sensitivity of this band with pH was used to determine the spectrophotometric apparent pK_a' (Fig. 10B and C). The average for all the individual pK_a values calculated was 10.44 ± 0.13 and was calculated by the linear regression method. The corresponding dissociation equilibrium is:



3.4. Analytical Studies

According to the obtained results by electrochemical and spectrophotometric studies, it was possible to apply these techniques to the quantitative analysis of tacrine. As working pH, pH 7.5

was selected both for DPP and UV techniques, because at this pH both signals, DPP peak and UV-vis bands are well resolved.

In order to provide a DPP quantitative procedure, the dependence between the tacrine concentration and peak current (I_p) was conducted. A linear relation in the concentration range between 5×10^{-6} and 1.6×10^{-4} M was found, indicating that the response was diffusion controlled in this range. Above this concentration (1.6×10^{-4} M) a loss of linearity was probably due to the adsorption of tacrine on the electrode.

On the other hand, with the UV spectrophotometric technique the concentration study was made at four absorbance bands (220, 240, 324 and 336 nm) and showed different linear response ranges and sensitivity (data not show). We selected the 324 nm band because it has a greater sensitivity and linearity than the others. The linearity was maintained between 5×10^{-6} and 7×10^{-4} M.

Based on the above results it was feasible to employ DPP and UV-vis techniques in the quantitative determination of the drug. For quantification we have used the calibration plot method for tacrine concentration ranging between 1.5×10^{-4} and 2.5×10^{-5} M at pH 7.5. The regression equation for each calibration plot was:

DPP $I_p(\mu A) = -19553 \times C(M) + 0.4 \quad (r = 0.9998, n = 10)$

UV-vis $A = 10393 \times C(M) - 3.1 \times 10^{-3} \quad (r = 0.9998, n = 10)$

The repeatability of the methods was adequate with variation coefficients of 1.2 % and 0.2 % for ten DPP and UV-vis measurements, respectively. From the reproducibility study (changing operator, equipment or both) the following variation was obtained, 1.3 % (DPP) and 0.2 % (UV-vis). Also the detection limits were determined obtaining a 4.8×10^{-6} M and 6.8×10^{-7} M values for DPP and UV respectively.

In order to obtain the precision and accuracy of the developed methods, we performed a recovery study for both techniques. The results are summarized in Table 1. These studies were made in presence of excipients but for the spectrophotometric studies a separation by centrifugation was necessary. These results reveal

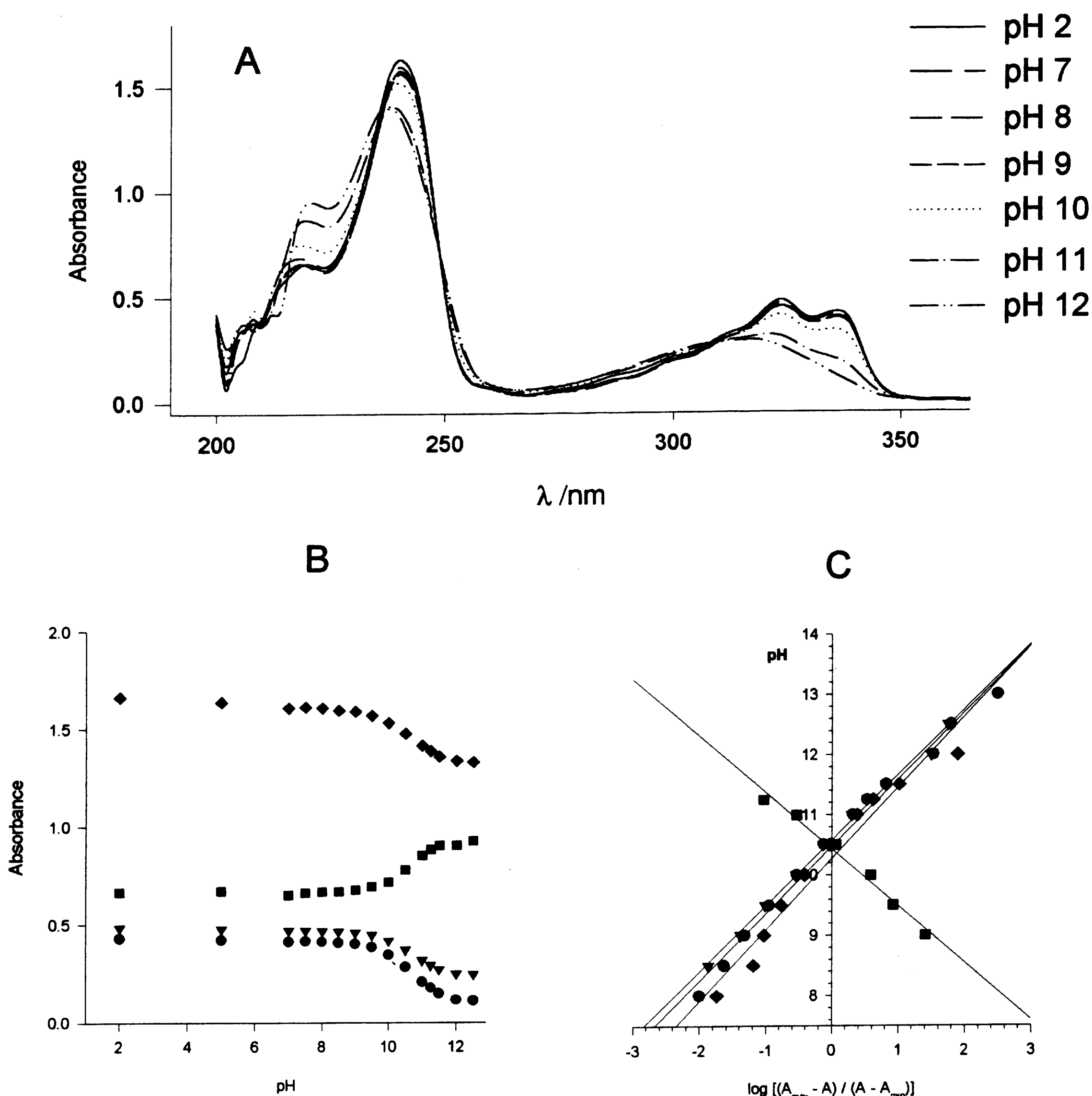


Fig. 10. UV spectra (A) of 0.05 mM tacrine solution at different pH and its pH dependence of the absorption maxima (B) and pK_a calculation plot (C). ●) 336, ■) 220, ▼) 324, ◆) 230 nm.

that both methods had adequate precision and accuracy and consequently can be applied to the determination of tacrine in pharmaceutical forms (capsules). The obtained results for the individual capsule assay (ICA) are shown in Table 2. From these results we can conclude that for both methods the requirements of uniformity content were fulfilled. In fact, the percentage of tacrine was found within the range 85–115% of the declared quantity and the variation coefficients were smaller than the 6% in agreement with the general criterion of the Pharmacopeia [22].

Considering both methods used in the analysis of tacrine we recommended the DPP technique as a good analytical alternative. The preparation of the sample was easy and since the excipients did not interfere in the electrochemical determination, separation was not necessary, as in the UV-vis method. Furthermore the analysis time was not long and the procedure had adequate precision and accuracy and consequently is strongly recommended for tacrine analysis in quality control laboratories or from samples taked at level of the pharmacy office.

4. Acknowledgements

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5. References

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