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Research Article

Determination of spectrophotometric protonation constant of cholinesterase inhibitors

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ABSTRACT

Spectrophotometric titration for protonation constant (pK_a) determination is one of the popular methods. In the present study pK_a values of rivastigmine, galantamine and donepezil which are cholinesterase inhibitors used in cure of Alzheimer's disease were determined using UV titration in various acetonitrile-water binary mixtures at constant temperature (25 °C) and ionic strength (0.1 mol l^{-1}). Data assessment was carried out with the STAR software program to calculate molar absorbance and stability constants of the compounds studied. Aqueous protonation constants of sparingly soluble drug compounds were calculated from two different extapolation methods. The extrapolated results are in good agreement with the literature values. Moreover, values of the protonation constant at 37°C, which are scarcely reported, were calculated by using Abraham's solute descriptors.

Keywords: Cholinesterase Inhibitors, Protonation Constant, UV-vis Spectrophotometry, Solute Descriptors

Kolinesteraz İnhibitörlerinin Spektrofotometrik Protonasyon Sabitinin Tayini

ÖZ

Protonasyon sabiti (pK_a) tayini için spektrofotometrik titrasyon popüler metotlardan birisidir. Sunulan çalışmada Alzheimer hastalığının tedavisi için kullanılan kolinesteraz inhibitörleri rivastigmin, galatamin ve donepezilin pK_a değerleri sabit sıcaklık (25 ^oC) ve iyonik şiddette (0,1 mol l^{-1}) farklı asetonitril-su ikili karışımlarında UV titrasyonla tayin edilmiştir. Veri değerlendirilmesi, çalışılan bileşiklerin kararlılık sabitleri ve molar absorbanslarının hesaplanması için kullanılan STAR yazılım programı ile yapılmıştır. Suda çözünmeyen ilaç bileşiklerinin sudaki protonasyon sabitleri iki farklı ekstrapolasyon yöntemi ile hesaplanmıştır. Ekstrapolasyon sonuçları literatür değerleri ile uyum içerisindedir. Ayrıca literatürde bulunmayan 37^{o}C' deki protonasyon sabiti değerleri Abraham çözünen parametreleri kullanılarak hesaplanmıştır.

Anahtar Kelimeler: Kolinesteraz inhibitörleri, Protonasyon sabiti, UV-vis spektrofotometri, Çözünen parametreleri

1. INTRODUCTION

Cholinesterase inhibitors are presently the most created cure strategy in Alzheimer's disease. This illness is a neurological disorder in which the death of brain cells causes cognitive decline and memory loss. Existing these inhibitors donepezil, rivastigmine and galantamine are widely recommended for clinical use.[1]

Protonation constant (pK_a) value of a molecule is an essential physicochemical parameter using in organic synthesis, medicinal chemistry, and material and food sciences.[2] The knowledge pK_a value of a molecule having acidic or basic properties help to predict ionization stage of this molecule at a given pH. This constant constitutes important data for agreement of absorption, distribution, metabolism, excretion and toxicology (ADMET).[3-6] Protonation equilibrium are usually determined in constant ionic strength. NaCl and KCl are usually used to maintain ionic strength. These chemicals are preferentially used in spectrophotometric and potentiometric titrations.

There are many various analytical techniques that allow for accurate investigation of pK_a value. Spectrophotometry is attractive method for pK_a determination because of several advantages such as specificity, rapidity, precision and accuracy. Moreover, this technique is a preferable method for providing foreknowledge to high performance liquid chromatography (HPLC). UV-vis spectrometry ensures the possibility of pK_a determination for compounds with simple equipment, low water solubility and sub-micromolar compound concentration. (about 10^{-5} to 10^{-6} M).[7,8] The compounds to be investigate in spectrophotometric pK_a determination should possess to pH-dependant light absorption. Some calculators using spectrophotometric data are often used for the estimation of pK_a.[9,10]

The effect of temperature on the equilibrium of chemical systems is well known. Calculation of temperature dependent pK_a is very important. The most reliable results come from laboratories where the pK_a is determined under standard conditions, i.e., in thermostated 25°C solutions containing a background electrolyte (e.g., 0.1 M KCl), with special care given to calibrating the pH electrode. Of the published pK_a values of drug molecules, scarcely any are reported at 37°C.[11] Data of the protonation constants at biorelevant temperature (37°C), which are barely information, are more significant for biological mechanisms of cellular transfer by ionizable compounds and in mechanistic disintegration works, which are frequently realized at 37°C.

The effect of temperature on pK_a depends on the nature of the functional group. Acidic drugs have nearly the same pK_a at 25 and 37°C, whereas basic drugs usually have a decreased pK_a at the biorelevant temperature ($pK_a/T \approx -0.03$ °C^{-1}).[11] In this work, the prediction of the pK_a value at 37 °C, provided the value at 25°C is known. For this, solute descriptors of Abraham and the pK_a value at 25°C were used in Eq. (1).[11]

$$\Delta pK_a = k_0 \, x \, pK_a^{25} + c_0 + c_1 \, x \sum \alpha_2^H + c_2 \, x \sum \beta_2^H + c_3 \, x \, \pi_2 + c_4 \, x \, R_2 + c_5 \, x \, V_x \quad (1)$$

Using calculated differences between the values (ΔpK_a), the pK_a value at 37°C is estimated by Eq. (2).[11]

$$\Delta pK_a = pK_a^{37} - pK_a^{25} \quad (2)$$

Evaluation of aqueous protonation constants is an inescapable requirement in practice drug development. However, many drugs are sparingly soluble in water and any experimental pK_a determination requires the use of cosolvent. The cosolvent procedure mainly using acetonitrile-water mixtures binary provides a good alternative for sparingly soluble compounds.[12-14]

A common approach for the pK_a measurement of aqueous insoluble compounds involves the use of different percentages of water-organic modifier binary mixtures and extrapolation to 0% water-organic modifier. Usually, at least three and up to six different percentages of water-organic modifier are recommended. There are two approach practised to estimate the aqueous protonation constant from the pK_a values determined in the hydroorganic mixture. In the first attitude, experimental protonation constant is drawn against mole fraction (X) of a hydroorganic mixture. This approach is performed by using Eq. (3).

$$pK_{a,\varphi} = aX + b \quad (3)$$

where a indicates the dissociation constant in water, X show the mole fraction of acetonitrile, b is the slope of the linear relationship, and $pK_{a,\varphi}$ is the pK_a at the corresponding composition. In second attitude, aqueous pK_a values are calculated by using Yasuda-Shedlovsky equation (Eq. 4). Based on the Born electrostatic model and Bjerrum's theory of ion association, Yasuda[15] and Shedlovsky[16] independently derived a correlation by means of a plot of $pK_a + \log [H_2O]$ versus $a\varepsilon^{-1} + b$, producing a straight line. Where $[H_2O]$ represents the molar water concentration and ε^{-1} denotes reverse of the dielectric permittivity of the binary mixture. Terms *a* and *b* symbolize the slope and the intercept of the plot, respectively.

$$pK_a + \log [H_2O] = a\varepsilon^{-1} + b \quad (4)$$

The results obtained for pK_a in water have been compared with those predicted by the Marvin Sketch program.[17] This program estimates several physico-chemical properties of compounds on the basis of their molecular structure.

In the present work, it was focused to investigate the protonation constants of rivastigmine, galantamine and donepezil. The determination of pK_a values at 25°C were carried out with spectrophotometric method in three different percentage acetonitrile (35%, 40% and 45%, v/v) for donepezil and rivastigmine, 25%, 30% and 35% (v/v) acetonitrile content for galantamine. Aqueous pK_a values of drugs studied were calculated with two different approaches. Moreover, the pK_a values at 37°C were estimated by applying Abraham's solute descriptors.

The main objective of our present work is to study the effect of cosolvents at fixed temperature and ionic strength on rivastigmine, galantamine and donepezil by using spectrophotometric method. Also, the extrapolated protonation constants results are to compare with those determined in aqueous medium or with literature values.

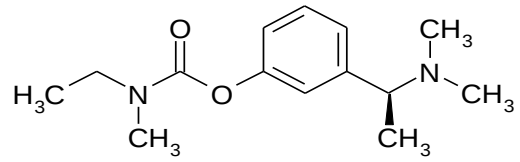
2. MATERIALS AND METHODS

2.1. Materials

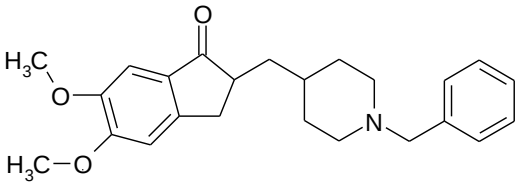
Galantamine hydrobromide and donepezil hydrochloride monohydrate were bought from Sigma-Aldrich (St. Louis, USA). Rivastigmine was supplied by Novartis Pharmaceuticals Corporation (Istanbul, Turkey). Potassium hydrogen phthalate (dried at 110 °C), potassium hydroxide using as a Titrisol, potassium chloride using as an ionic strength adjuster, and acetonitrile (HPLC grade) using as a cosolvent were provided by Merck (Darmstadt, Germany). All of the chemicals used in this work were utilized without any further purification. Solutions and solvent mixtures were prepared with distilled water obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system. Chemical structures of donepezil, rivastigmine and galantamine are given in Figure 1.

2.2. Methods

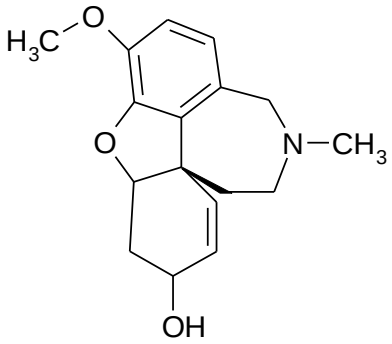
In this study, pH measurements were carried out with a In Lab 412 glass electrode, using a Mettler Toledo MA 235 pH/ion analyser (Schwerzenbach, Switzerland).



Rivastigmine



Donepezil



Galantamine

Figure 1. Structure of rivastigmine, donepezil and galantamine.

These measurements were actualized at 25°C. Potassium hydrogen phthalate (0.05 mol kg^{-1}) was used as primary standard buffer reference solution for the standardization of this apparatus in acetonitrile-water mixtures in accordance with IUPAC rules.[18]

For the determination of the protonation constants of selected drugs, 35%, 40% and 45% (v/v) water-acetonitrile binary mixtures for donepezil and rivastigmine and 25%, 30% and 35% (v/v) water-acetonitrile binary mixtures for galantamine in 0.1 mol l^{-1} KCl (ionic strength) adjusted water were prepared and used throughout experimental investigation.

The spectrophotometric pH-titration was performed in hydroorganic mixtures containing 1 x 10^{-6} mol l^{-1} drug and 0.1 mol l^{-1} KCl solution for adjustment of an ionic strength. In each experiment, drug samples were titrated with 0.05 mol l^{-1} KOH to an appropriately high pH, usually 11.7. Titrations were performed at 25°C and fixed ionic strength (0.1 mol l^{-1} KCl).

The attained spectrum was set down using UV-visible spectrophotometry (Lambda 25, Perkin Elmer, USA). All titrations were performed with small volume plus (0.05 ml). Temperature of titration conditions was fixed at 25°C ± 0.1 by way of a cooler system water bath (Heto CBN 8-30). Spectral data were obtained in the pH range of 5.8-11.7. When the electromotive force (emf) was stable, the spectra of the drugs were recorded with 200 to 400 nm interval for rivastigmine, donepezil and galantamine.

Spectrophotometric protonation constants were calculated using the STAR program (stability constants by absorbance readings).[19] The program refines the absorbance values, until a minimum value in the sum of squared differences between the experimental (A_{exp}) and calculated absorbance (A_{calc}) for each data point. The minimization process is repeated until the relative change of the sum of the squares residual (U) between two iterations is 0.01%.

3. RESULTS AND DISCUSSION

Investigation of pK_a values of the studied basic inhibitors were performed with the UV-spectrophotometric titration measurement in the same acetonitrile-water hydroorganic mixtures to prove the verification of pK_a values obtained in previous work[20] (35%, 40% and 45% (v/v) water-acetonitrile binary mixtures for donepezil and rivastigmine; 25%, 30% and 35% (v/v) water-acetonitrile binary mixtures for galantamine). Spectral data of drugs investigated were obtained from absorbance measurements in 200 to 400 nm interval in the pH range of 5.8-11.7, and they were depicted in Figure 2 and 3. The pK_a values evaluated by STAR program using the obtained data for drugs studied at 25°C are given with their standard deviations in Table 1. Typical spectrophotometric titration curves obtained from absorbance measurements at 230 nm for donepezil and galantamine and 225 nm for rivastigmine in acetonitrile-water at 35% volume fraction at 25°C are shown in Figure 4. These sigmoidal curves were obtained from NLREG program.[21]

As a continuation of this determination, the pK_a values at 37°C were predicted using the experimental pK_a values at 25°C. The differences between the values (ΔpK_a) were calculated with Abraham's solute descriptors. According to the values of the Abraham descriptors, small amounts of hydrogen bonding cause pK_a to take on more negative values. The average pK_a in the bases is -0.283; the values range from -0.266 (rivastigmine pK_{a2}) to -0.296 (donepezil and galantamine). Calculated data at 37°C were also compared with experimental data (Table 1).

The studied drugs have one pK_a value corresponding to base functional group. As acetonitrile concentration increases, the dissociate capabilities of basic inhibitors decrease and lead to reduce the value of pK_a. The pK_a values at 37°C of these inhibitors were determined here for the first time. Aqueous pK_a values were calculated for sparingly soluble drug compounds. In the first approach, pK_a values were plotted against to acetonitrile mole fraction. The intercepts of these linear equations obtained from Eq. (3) are the aqueous pK_a values of these compounds.

Table 1. The pK_a values determined by spectrophotometric titration in acetonitrile effect and predicted pK_a values acetonitrile-water binary mixtures

Compounds	Experimental pK_a (25 °C)			ΔpK_a (calculated)			Predicted pK_a (37 °C)		
	35% (v/v)	**40% (v/v)**	**45% (v/v)**	**35% (v/v)**	**40% (v/v)**	**45% (v/v)**	**35% (v/v)**	**40% (v/v)**	**45% (v/v)**
Donepezil	8.02±0.03	7.78±0.27	7.54±0.03	-0.296	-0.289	-0.283	7.727	7 492	7.256
Rivastigmine	7.41±0.06	7.17±0.05	6.89±0.09	-0.280	-0.274	-0.266	7.137	6.900	6.626
	25% (v/v)	**30% (v/v)**	**35% (v/v)**	**25% (v/v)**	**30% (v/v)**	**35% (v/v)**	**25% (v/v)**	**30% (v/v)**	**35% (v/v)**
Galantamine	8.04±0.13	7.68±0.08	7.33±0.03	-0.296	-0.287	-0.278	7.744	7.400	7.052

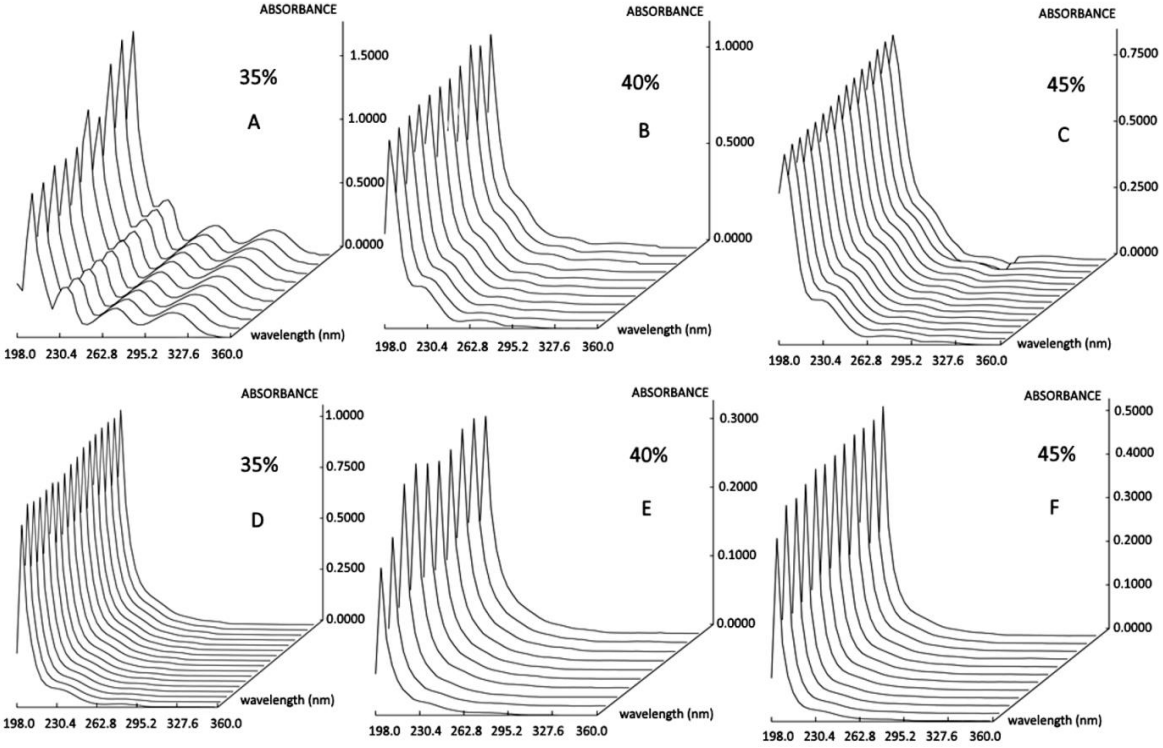


Figure 2. The wavelength (nm)-absorbance graphs for donepezil (1) and rivastigmine (2) in a) 35% (v/v) , b) 40% (v/v). c) 45% (v/v) acetonitrile-water binary mixtures.

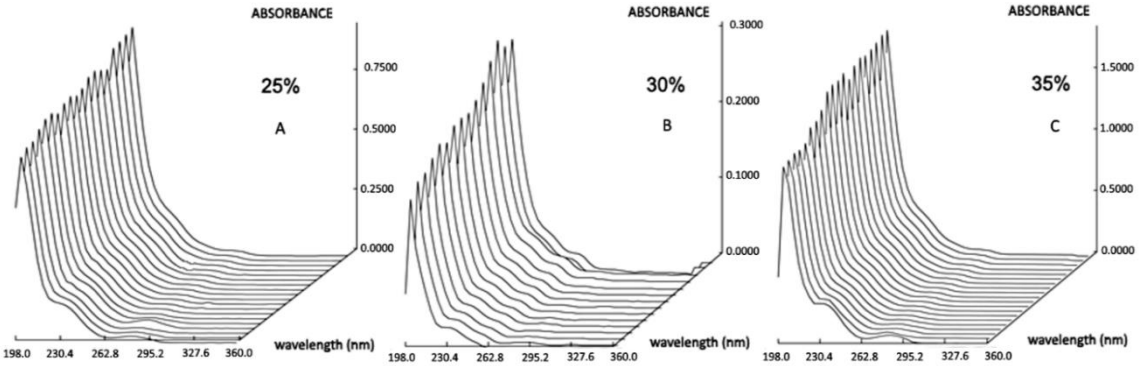


Figure 3. The wavelength (nm)-absorbance graphic for galantamine in a) 25% (v/v) , b) 30% (v/v), c) 35% (v/v).

Regression analysis results are listed in Table 2. These results indicate that, in general, the assumption of a linear relationship between $pK_{a,\varphi}$ and mole fraction of acetonitrile is a good approximation within studied interval (25-45%, v/v).

Table 2. The linear equations data obtained from relationship of acetonitrile mole fraction and $pK_{a,\varphi}$ values

Compounds	Equations	r
Donepezil	$pK_{a,\varphi}$ = -8.651(0.045) + 9.311(0.008)	0.999
Rivastigmine	$pK_{a,\varphi}$ = -9.387(0.213) + 8.819(0.038)	0.999
Galantamine	$pK_{a,\varphi}$ = -14.379(0.222) + 9.471(0.028)	0.999

In second approach, Yasuda-Shedlovsky equation (Eq. 4) was used in order to predict aqueous pK_a values using the pK_a values obtained from STAR programme (Table 3). The linearity of the plots is characterized by the regression coefficients (r^2) values which indicate significant linear correlation for the molecules examined drugs. It can be seen from the plots that bases have negative slopes (Figure 5) and produce straight lines with randomly scattered points the total interval (ε: 60.109-68.941). As listed in Table 5, the agreement between the pK_a values of the samples obtained from various acetonitrile-water mixtures and literature values is generally good.

Table 3. Data obtained from Yasuda-Shedlovsky extrapolation

Compounds	ACN (v/v%)	ε	$pK_a + \log[H_2O] = a\varepsilon^{-1} + b$		r	N
			a (slope)	*b* (intercept)		
Donepezil	35	64.563	-426.0	16.35	1.000	3
	40	62.331				
	45	60.109				
Rivastigmine	35	64.563	-461.9	16.30	0.999	3
	40	62.331				
	45	60.109				
Galantamine	25	68.941	-706.0	20.00	0.999	3
	30	66.770				
	35	64.563				

*a and b are empirical fitting constants, ε^{-1} is the reverse of the dielectric permitivity of the binary solvent, r is correlation coefficient, log[H_2O] = log 55.5 is molar concentration of pure water, N is number of values.

Table 4. Comparison of aqueous pK_a values of cholinesterase inhibitors

	Experimental aqueous pK_a (25°C)		Predicted aqueous pK_a (37 °C)		Our previous work[20] (30 °C)		Our previous work[22] (30 °C)		Literature values	Marvin Sketch[17]
	Yasuda-Shedlovsky	pKa-X	Yasuda-Shedlovsky	pKa-X	Yasuda-Shedlovsky	pKa-X	Yasuda-Shedlovsky	pKa-X		
Donepezil	9.166	9.311	8.835	8.967	8.534	8.555	8.892	8.555	9.10[26]	8.62
Rivastigmine	8.657	8.819	8.347	8.493	9.019	9.035	8.771	9.085	8.90; 8,9[24,25]	8.89
Galantamine	9.239	9.470	8.919	9.138	8.493	8.571	8.269	8.340	8.21[23]	8.91

20. Reversed phase liquid chromatography determination at 30°C and linear correlation method to obtain the pK_a value
22. Central composite design at 30°C (predicted)
23. Spectrophotometric determination in water at 25°C
24. No estimated uncertainty quoted
25. Radial basis function neural networks and the heuristic method (calculation)
26. Capillary electrophoresis determination at 25°C and ionic strength of 0.05 M

A further comparison between the pK_a values calculated in aqueous medium and those given in the literature and predicted by Marvin Sketch shows that the values corresponding to the pK_a are generally in good agreement. The remarkably good results obtained by this program should be mentioned, because predicted values are within 0.2-0.5 pKa units from real experimental values.

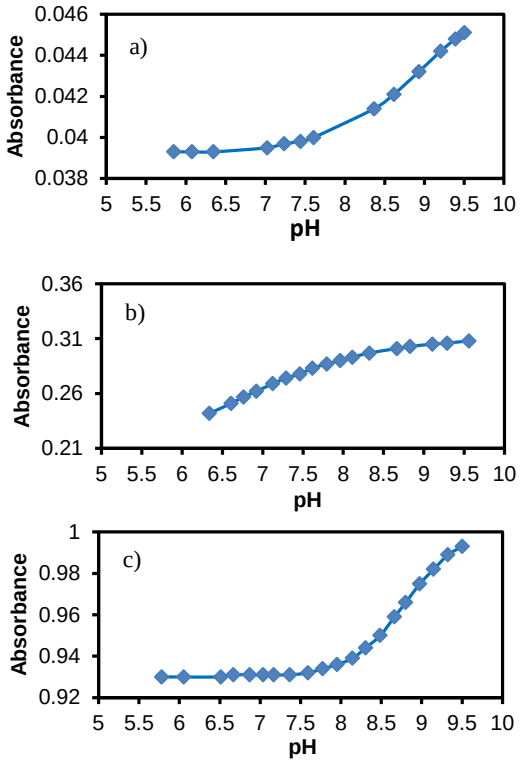


Figure 4. The relationship between A and pH obtained from absorbance measurements in acetonitrile-water at 35% volume fraction at 25°C. a) Donepezil, b) Rivastigmine, c) Galantamine.

Aqueous protonation constant values obtained from two approaches were given in Table 4. Aqueous pK_a values at 37°C were predicted using experimental aqueous pK_a values. These experimental and predicted aqueous pK_a values were compared with pK_a values given from our previous works[20,22] and the other works reported[23-26] (Table 4). In literature reports, different pK_a values for the same compounds are calculated. In the present study, our results[20,22] are compatible with each other. These calculated values were obtained by using different methods at the same temperature. Investigated compounds were estimated by Demiralay and co-workers[22] in the binary mixtures of acetonitrile-water in the same concentrations by the experimental design method. The calculated pK_a values for galantamine are not in full consistency with that reported by Meloun and co-workers.[23] In that study, galantamine was determined in different ionic strengths and temperatures. For rivastigmine, pK_a values were predicted by Hsieh and co-workers[24] and Luan and co-workers.[25] In these studies, there are no experimental values. There is only one study for donepezil in the literature.[26] This study was carried out by capillary electrophoresis method. Therefore, the calculated pK_a values for donepezil are not in full consistency with that reported by Ishihama and co-workers.[26] Table 4 shows that the close values are obtained in determining protonation constants, despite of the use of different equipment by different researchers on different times for different drug concentrations. A comparison with representative pK_a values available in the literature shows that either method gives satisfactory values.

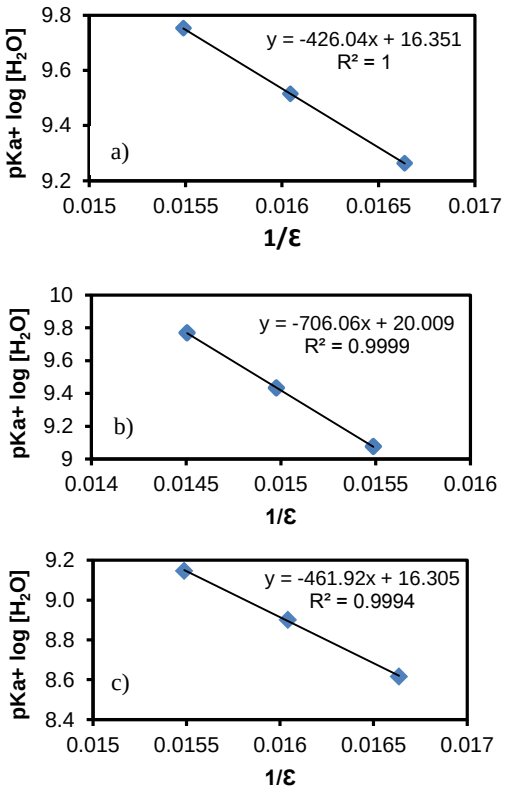


Figure 5. The Yasuda-Shedlovsky plots of a) Donepezil, b) Rivastigmine, c) Galantamine.

4. CONCLUSİON

In this study, the pK_a values of galantamine, donepezil and rivastigmine which are used in the cure of Alzheimer's disease were determined by using spectrophotometric titration in acetonitrile-water binary mixtures. pK_a values at 37°C were predicted with Abraham solute descriptors. So, this data is preliminary in the literature. There was of enormous consistence between the aqueous pK_a values of the studied drugs by calculated values and predicted values. The significant data obtained from this study can be used for pharmacological, pharmacokinetic works of cholinesterase inhibitors.

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Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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