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Research Article

CALCULATİON OF ACİD DİSSOCİATİON CONSTANTS AND BİOLOGİCAL ACTİVİTİES İN SOME PYRİMİDİNE DERİVATİVES AS KNOWN DRUG ACTİVE SUBSTANCE

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ABSTRACT

Acid dissociation constants (pKa) of some pyrimidine derivatives were determined experimentally with potentiometric titration method. All compounds were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents (isopropyl alcohol, N,N-dimethyl formamide, tert-butyl alcohol and acetonitrile). Thus, the half-neutralization potential values and the corresponding pKa values were determined in all cases. In addition, pKa were determined theoretically with SPARC computer program. Pyrimidine derivatives have known different structures as drug active substance were evaluated for their biological activities such as antimicrobial activities.

Keywords: Acid dissociation constants, potentiometric titration method, SPARC computer program

INTRODUCTION

Pyrimidines are very important six member heterocyclic containing two nitrogen atoms. Pyrimidines are present among the three isomeric diazines.[1] Several (mainly uracil, thymine and cytosine) pyrimidines have been isolated from the nucleic acid hydrolyses. The nucleic acid are essential constituent of all cells and thus of all living matter cytosine is found to be present in both types of nucleic acids i.e. ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) while uracil present only in RNA and thymine only in DNA.[2] In addition to this, pyrimidines ring is also found in vitamin B1, barbituric acid (2, 4, 6-trihydroxy pyrimidine) and its several derivatives e.g. Veranal which are used as hypnotics.[3]

Compound having pyrimidine nucleus possesses broad range of biological activity as anticancer, antiviral, anti-HIV,antibacterial, antihypertensive, anticonvulsant, antithyroid and H1-antihistaminics antibiotics.[4] As a result of remarkable pharmacological activity of pyrimidine derivatives, intensive research has been made focused on biological activity.[1]

The acid dissociation constant(pKa) represents an important parameter for evaluation of the acid–base properties of pyrimidine and for estimation of their properties in solvent environment. The acid dissociation constant (pKa) of the weak base provides information about a form of the compound (neutral or protonated) presentina solution.[5] The knowledge of pKa of weak electrolytes is important also in analytical chemistry in optimization of experimental conditions for separation and analysis of many types of compounds.[6,7] On the other hand, the acid dissociation constant, usually expressed as its pKa value, is one of the most important parameters describing physicochemical properties of drugs.[8,9] It characterizes acid–base equilibrium of the respective ionizable groups in solution, thus, indicates their dissociation (deprotonation) potential at a given pH.[10] The acidic and basic forms of drugs differ in charge – at least one of them is ionized, therefore, they also may differ in other key properties like water solubility.[10]

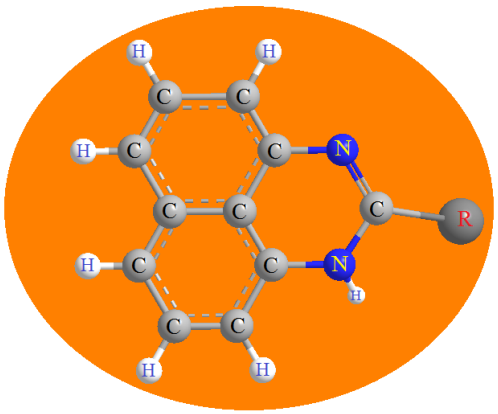
EXPERIMENTAL SECTION

Acidity

Potentiometric Titration

In this study, twelve different pyrimidine derivatives ((**1**) 2-benzyl-1*H*- pyrimidine, (**2**) 2-(3-methylbenzyl)-1*H*- pyrimidine, (**3**) 2-(2-fluorobenzyl)-1*H*- pyrimidine, (**4**) 2-(3-fluorobenzyl)-1*H*- pyrimidine, (**5**) 2-(4-

fluorobenzyl)-1*H*- pyrimidine, (**6**) 2-(2-bromobenzyl) -1*H*-pyrimidine, (**7**) 2-(3-bromobenzyl)-1*H*-pyrimidine, (**8**) 2-(4-bromobenzyl)-1*H*- pyrimidine, (**9**) 2-(2-chlorobenzyl)-1*H*- pyrimidine, (**10**) 2-(2,6-dichlorobenzyl)-1*H*- pyrimidine, (**11**) 2-(3,4,5-trimethoxybenzyl)-1*H*-pyrimidine, (**12**) 2-((4-chlorophenoxy) methyl-1*H*- pyrimidine) weresynthesized in Recep Tayyip Erdoğan University Organic Chemistry Research Laboratory and published.[11] This pyrimidine derivatives were titrated with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents (isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile), using potentiometric method.



Molecule	-R	Molecule	-R
1	-benzyl	7	-3-bromobenzyl
2	-3-methylbenzyl	8	-4-bromobenzyl
3	-2-fluorobenzyl	9	-2-chlorobenzyl
4	-3-fluorobenzyl	10	-2,6-dichlorobenzyl
5	-4-fluorobenzyl	11	-3,4,5-trimethoxybenzyl
6	-2-bromobenzyl	12	-(4-chlorophenoxy)methyl

Fig. 1: General molecule formüle for studied pyrimidine derivatives

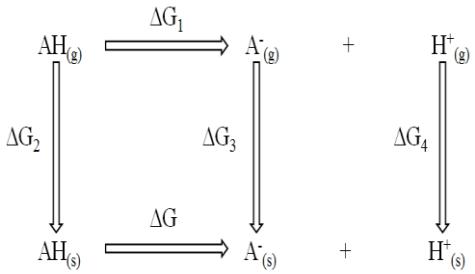
Orion Model 720A pH ion meter, fitted witha combined pH electrode (Ingold) was used for potentiometric titrations. An Ingold pH electrode was preferred because of the advantage. A magnetic stirrer, a semi-micro burette and a 25 mL beaker were also used in titrations. All the chemicals were supplied from Merck. Before potentiometric titrations (Figure 2), the pH meter was calibrated according to the instructions supplied by the manufactures of the pH meter. In this section, the pH electrode calibrated with 4, 7, 10 and 12 pH tampon solution.[12]

Fig. 2: System of potentiometric titration cell used in studied

For each compound that would be titrated, the 0.001 M solution was separately prepared in each non-aqueous solvent (isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile). During the titrations, the titrant was added in increments of 0.05 mL after each stable reading, and mV values were recorded. After purifications, isopropyl alcohol was used to prepare 0.05 N tetrabutylammonium hydroxide (TBAH). For all potentiometric titrations, 0.05 N TBAH in isopropyl alcohol, which was prepared from 0.1 N TBAH by dilution, was used. The mV values, that were obtained in pH meter, were recorded. Graphs were drawn by obtained from all data and end point is determined by $\Delta E/\Delta V$ - (TBAH, mL), $\Delta^2E/\Delta V^2$ - (TBAH, mL) and $\Delta V/\Delta E$ - (TBAH, mL) graphics. Finally, the half-neutralization potential (HNP) values were by drawing these graphic and pKa values were determined according to half-neutralization method.[12]

SPARC Computer Program

The computer program SPARC (SPARC Performs Automated Reasoning in Chemistry) was developed to predict numerous physical properties such as vapor pressure, distribution coefficient, and GC retention time as well as chemical reactivity parameters such as pKa and electronaffinity. SPARC predicts both macroscopic and microscopic pKa values strictly from molecular structure using relatively simple reactivity models SPARC computer program is based on the thermodynamic cycle (Figure 3) as shown below.[13]



$\Delta G = [((\Delta G_3 + \Delta G_4) - \Delta G_2) + \Delta G_1]$
$\Delta G = -2.303 \cdot R \cdot T \cdot \log Ka$

Fig. 3: Thermodynamic cycle and calculation of Gibbs Free Energy

The ionization of weak acid (HA) is given for the gas and solvent phase in Figure 3. Calculation of pKa were made using the free energy changes in the thermodynamic cycle. Respectively ΔG_1, ΔG_2, ΔG_3 and ΔG_4 are calculated for find the ΔG (in solvent phase). Then, pKa is calculated using the equation with calculated ΔG. In this paper, we describe the details of the SPARC reactivity computational methods and its performance on predicting the pKa values of these benzimidazole derivatives in comparison with experimental values.[12]

Antimicrobial

All microorganisms used in the study were obtained from the Hıfzıssıhha Refik Saydam Institute in Ankara (Turkey). Microorganisms, *Escherichia coli* ATCC 25922, *Bacillus cereus* 702 Roma, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 43288, *Yersinia pseudotuberculosis* ATCC 911, Staphylococcus aureus ATCC 25923, Listeria monocytogenes ATCC 43251, *Mycobacterium smegmatis* ATCC 607 were used as bacteria and strain *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251 were used as ferment in the study. All chemicals were dissolved in DMSO at 20 mg/mL concentrations.

Agar Well Diffusion Method

An agar well diffusion method[14,15] was used to measure the antimicrobial activities of the pyrimidine derivatives. Dilutions of the bacteria to be tested were prepared approximately 10^6 cfu/mL (colony forming unit) in Mueller Hinton liquid medium (MHB) after from one night culture. They were planted on previously prepared MH agar medium. Yeast dilution were prepared used extract liquid medium (YE) approximately 10^7 cfu/mL and pre-prepared potato dextrose agar (PDA) was planted in the media. Wells were opened 2 cm spacing and 5 mm diameter on the mediums that had been planted by the help of sterile glass tube. 50 µL was dropped each wells from chemical stock solutions. The petri dishes containing bacteria for 24 hours, the petri dishes containing yeast for 48 hours were incubated at 35°C. Zone diameters blocked by uremeni were measured by the help of ruler after incubation. Ampicillin (10 µg/mL) was used as the standard control drug, Fluconazole (5 µg/mL) was used for the yeast and DMSO was used as the solvent control.

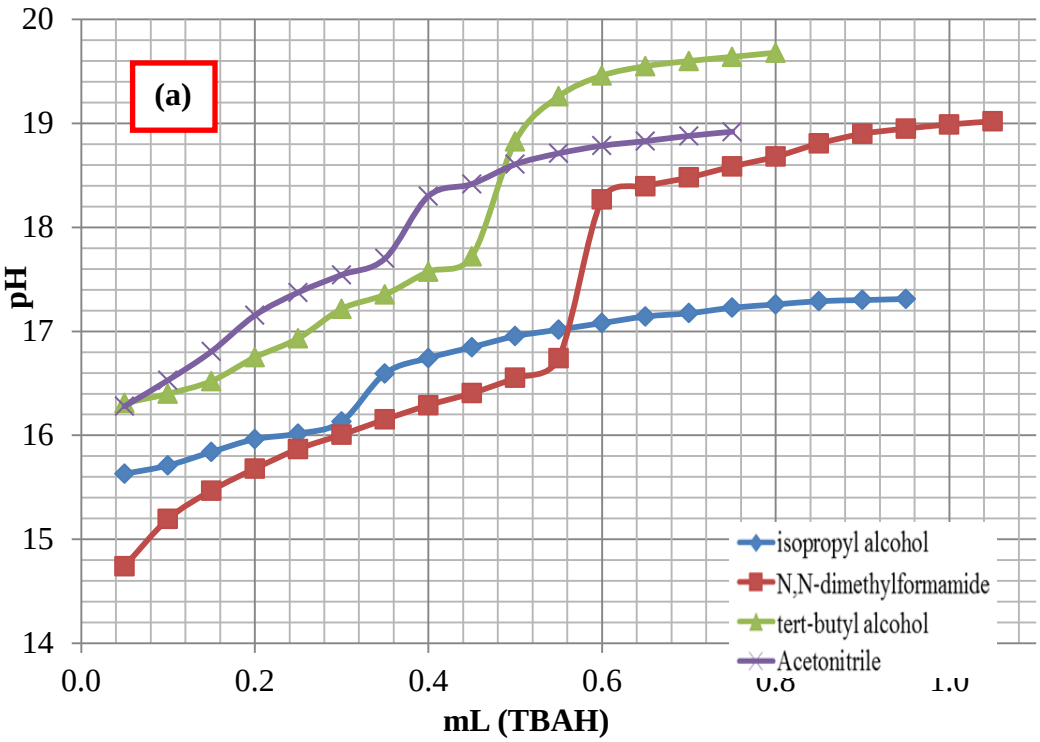
Minimal Inhibition Concentration Method

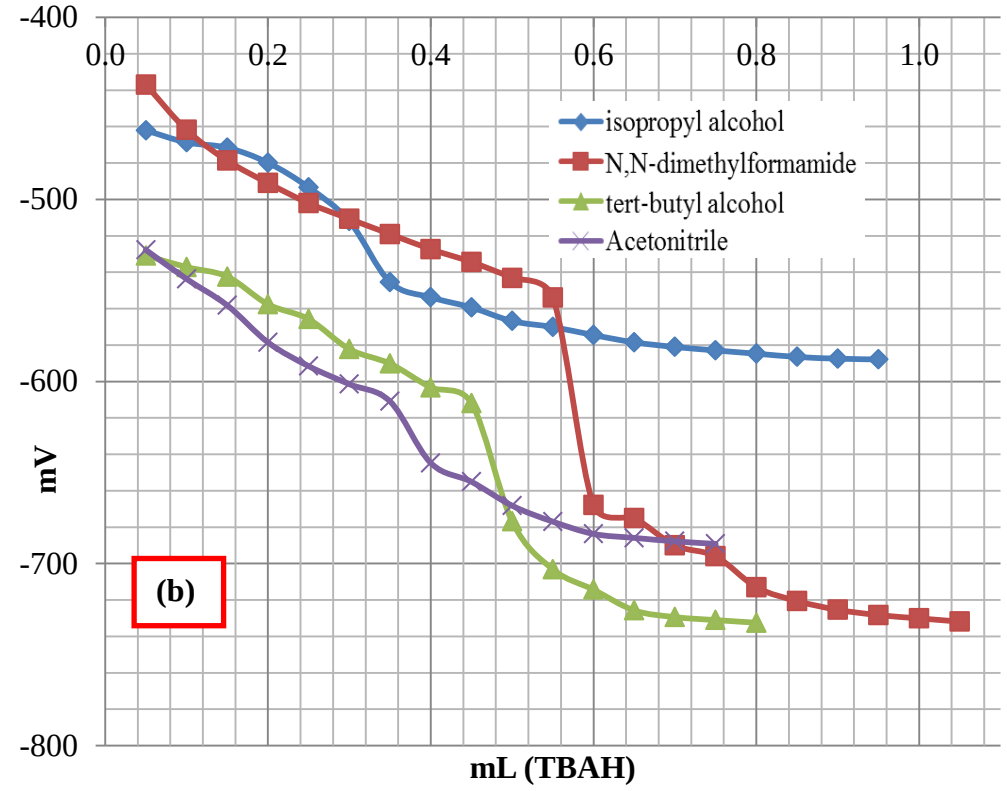
Minimal inhibitory concentration (MIC) values (µg/mL) of the pyrimidine derivatives were determined on microplate using microdilution method. Antibacterial tests were performed on medium of Mueller-Hinton Broth (MHB) (pH 7.3), antifungal activity tests were performed on medium of buffered Yeast Nitrogen Base (pH 7.0). The minimum inhibitory concentration value was determined as the concentration of the substance in the well that was not developed. Ampicillin (10 µg), Streptomycin (10 µg) and Fluconazole (5 µg) were used as standard antimicrobials.

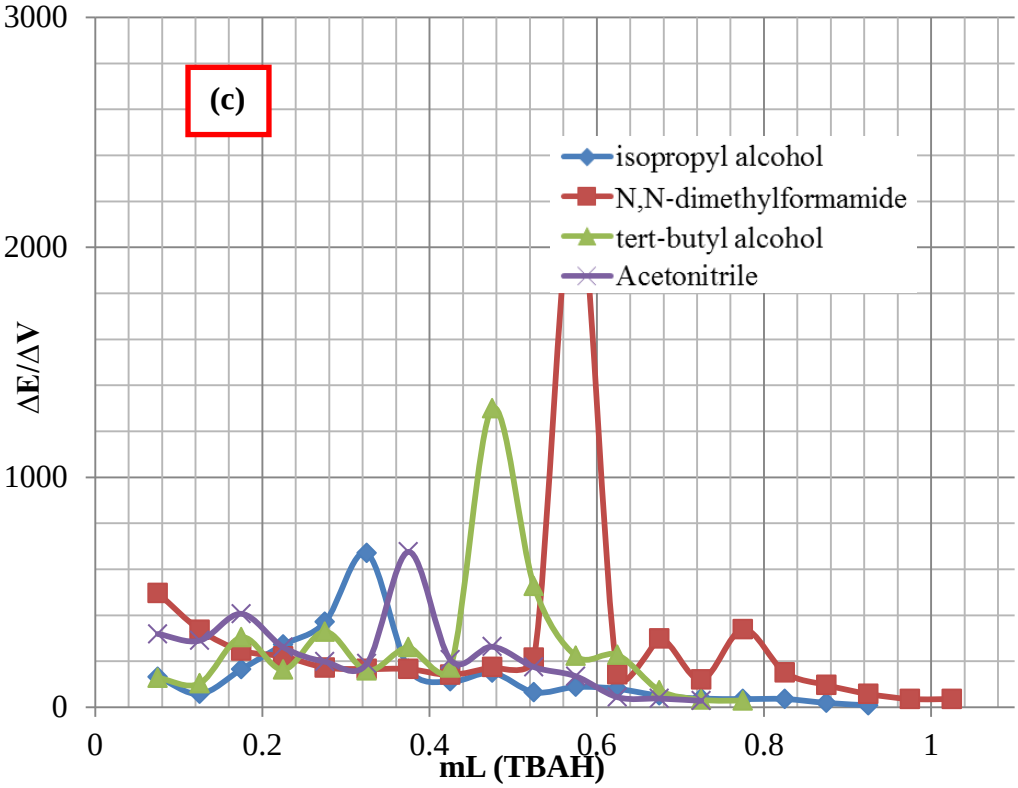
RESULTS AND DISCUSSION

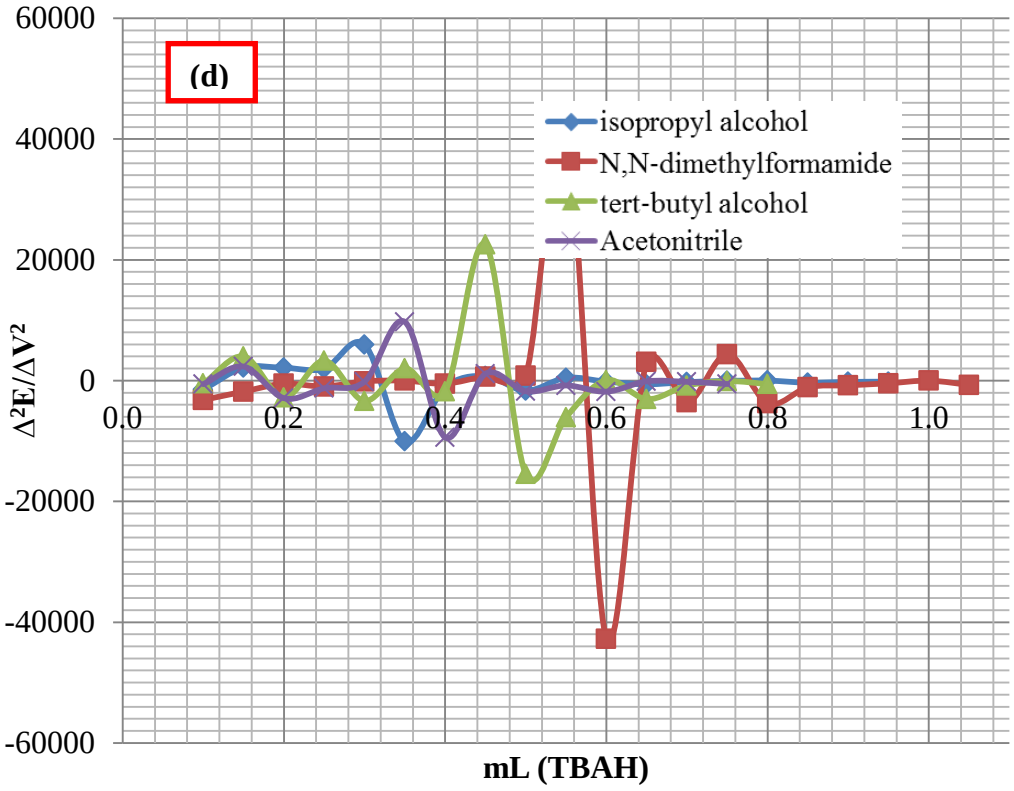
Acidity

In this study, pyrimidine derivatives were titrated potentiometrically with TBAH in isopropyl alcohol, *N,N*-dimethylformamide, *tert*-butyl alcohol and acetonitrile. The mV values read in each titration were drawn against TBAH volumes (mL) added and potentiometric titration curves were formed for all the cases. Experiments were repeated 3 times in each experiment. Standard deviations was calculated for this three experiments. Calculations were performed within 95 % confidence interval. From the titration curves (Figure 4), the HNP (half-neutralization potential) values were measured and the corresponding pKa values were calculated. The HNP values and the corresponding pKa values of all triazole derivatives, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *N,N*-dimethyl formamide, *tert*-butyl alcohol and acetonitrile and pKa for all compounds were calculated theoretically with SPARC computer programme. All pKa (experimental and theoretical), HNP and percentage relative error values (between the theoretical values and the experimental values) are given in Table 1. Theoretical and experimental pKa values were comparisoned as an example of the compound **1** in Figure 5.[12]




-400
-500
-600
-700
-800
0.0
0.2
0.4
0.6
0.8
1.0
mV
mL (TBAH)
isopropyl alcohol
N,N-dimethylformamide
tert-butyl alcohol
Acetonitrile
(b)


3000
2000
1000
0
0
0.2
0.4
0.6
0.8
1
ΔE/ΔV
mL (TBAH)
isopropyl alcohol
N,N-dimethylformamide
tert-butyl alcohol
Acetonitrile
(c)



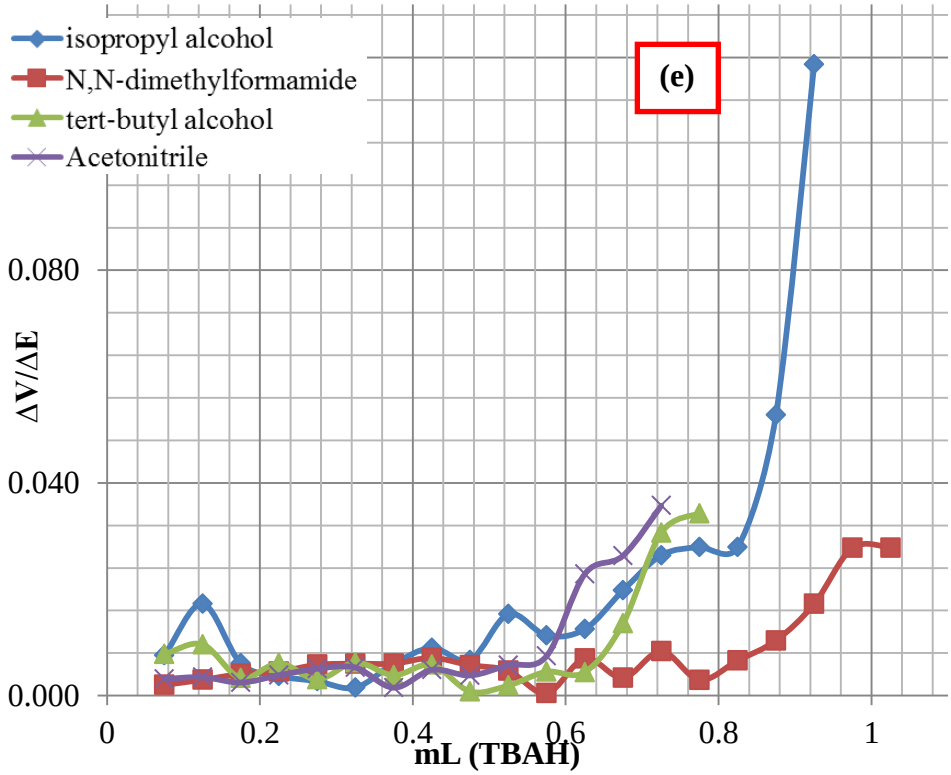


Fig. 4: (a) pH-mL, (b) mV-mL, (c) ΔE/ΔV-mL, (d) Δ²E/ΔV²-mL and (e) ΔV/ΔE-mL potentiometric titration curves of compound 1 at 25 °C.

Table 1: Half-Neutralization Potential (HNP) Values, experimental and theoretical the corresponding pKa values with percentage relative error values of all studied molecules

Comp.	Solvent	pKa (Experimental)	HNP (mV)	pKa (Theoretical)	Relative Error, %
1	Isopropyl alcohol	15.88 ± 0.14	-473.8 ± 6.8	18.60	-17.13
	N,N-Dimetilformamid	15.96 ± 0.12	-507.9 ± 8.2	18.11	-13.47
	Tert-Butily alcohol	16.87 ± 0.10	-562.7 ± 7.5	20.84	-23.53
	Acetonitrile	17.04 ± 0.11	-572.1 ± 7.3	21.02	-23.36
2	Isopropyl alcohol	15.84 ± 0.12	-474.3 ± 6.8	18.76	-18.43
	N,N-Dimetilformamid	15.89 ± 0.06	-505.5 ± 4.7	18.28	-15.04
	Tert-Butamol	16.94 ± 0.13	-568.0 ± 7.9	20.01	-18.12
	Asetonitril	17.01 ± 0.10	-572.2 ± 8.7	21.19	-24.57
3	Isopropyl alcohol	16.57 ± 0.11	-545.1 ± 7.4	18.40	-11.04
	N,N-Dimetilformamid	15.96 ± 0.09	-507.7 ± 6.6	17.92	-12.28
	Tert-Butily alcohol	17.44 ± 0.14	-598.9 ± 9.1	20.65	-18.41
	Acetonitrile	16.52 ± 0.08	-545.4 ± 5.9	20.83	-26.09
4	Isopropyl alcohol	15.43 ± 0.13	-465.0 ± 4.8	18.44	-19.51
	N,N-Dimetilformamid	16.58 ± 0.12	-544.2 ± 7.3	17.96	-8.32
	Tert-Butily alcohol	17.23 ± 0.10	-588.7 ± 6.7	20.68	-20.02
	Acetonitrile	17.61 ± 0.14	-606.8 ± 8.1	20.87	-18.51
5	Isopropyl alcohol	15.63 ± 0.08	-486.2 ± 8.8	18.45	-18.04
	N,N-Dimetilformamid	16.45 ± 0.13	-538.5 ± 6.5	17.97	-9.24
	Tert-Butily alcohol	16.42 ± 0.07	-537.4 ± 7.3	20.70	-26.07
	Acetonitrile	17.17 ± 0.12	-581.6 ± 4.8	20.88	-21.62
6	Isopropyl alcohol	16.32 ± 0.11	-530.2 ± 6.7	18.36	-12.50
	N,N-Dimetilformamid	16.70 ± 0.14	-551.6 ± 7.7	17.87	-7.01
	Tert-Butily alcohol	16.99 ± 0.09	-572.3 ± 8.4	20.61	-21.31
	Acetonitrile	16.39 ± 0.11	-534.0 ± 5.6	20.79	-26.85
7	Isopropyl alcohol	16.42 ± 0.10	-535.7 ± 8.8	18.39	-12.00
	N,N-Dimetilformamid	15.71 ± 0.12	-411.9 ± 6.9	17.90	-13.94
	Tert-Butily alcohol	15.53 ± 0.08	-397.9 ± 4.3	20.63	-32.84
	Acetonitrile	15.89 ± 0.14	-421.3 ± 6.5	20.82	-31.03
8	Isopropyl alcohol	15.68 ± 0.13	-409.2 ± 7.5	18.40	-17.35
	N,N-Dimetilformamid	15.10 ± 0.07	-375.3 ± 9.3	17.91	-18.61
	Tert-Butamol	16.30 ± 0.09	-447.7 ± 7.6	20.64	-26.63
	Asetonitril	15.83 ± 0.11	-499.9 ± 5.9	20.83	-31.59
9	Isopropyl alcohol	16.46 ± 0.08	-539.8 ± 5.5	18.35	-11.48
	N,N-Dimetilformamid	15.80 ± 0.10	-489.2 ± 8.6	17.86	-13.04
	Tert-Butily alcohol	17.44 ± 0.11	-600.6 ± 9.1	20.60	-18.12
	Acetonitrile	16.57 ± 0.07	-547.0 ± 4.9	20.78	-25.41
10	Isopropyl alcohol	16.18 ± 0.10	-529.6 ± 9.5	18.12	-11.99
	N,N-Dimetilformamid	15.66 ± 0.13	-534.9 ± 4.8	17.63	-12.58
	Tert-Butily alcohol	16.82 ± 0.08	-572.3 ± 6.7	20.36	-21.05
	Acetonitrile	16.01 ± 0.11	-519.6 ± 6.1	20.55	-28.36
11	Isopropyl alcohol	15.18 ± 0.10	-421.8 ± 9.7	18.06	-18.97
	N,N-Dimetilformamid	15.44 ± 0.13	-436.5 ± 6.8	17.57	-13.80
	Tert-Butily alcohol	15.46 ± 0.08	-484.5 ± 8.7	20.31	-31.37
	Acetonitrile	16.58 ± 0.08	-526.0 ± 7.5	20.50	-23.64
12	Isopropyl alcohol	14.65 ± 0.06	-407.1 ± 5.3	18.00	-22.87
	N,N-Dimetilformamid	15.03 ± 0.08	-429.5 ± 8.6	17.52	-16.57
	Tert-Butily alcohol	15.00 ± 0.10	-471.7 ± 7.6	20.25	-35.00
	Acetonitrile	15.83 ± 0.09	-502.1 ± 8.2	20.43	-29.06

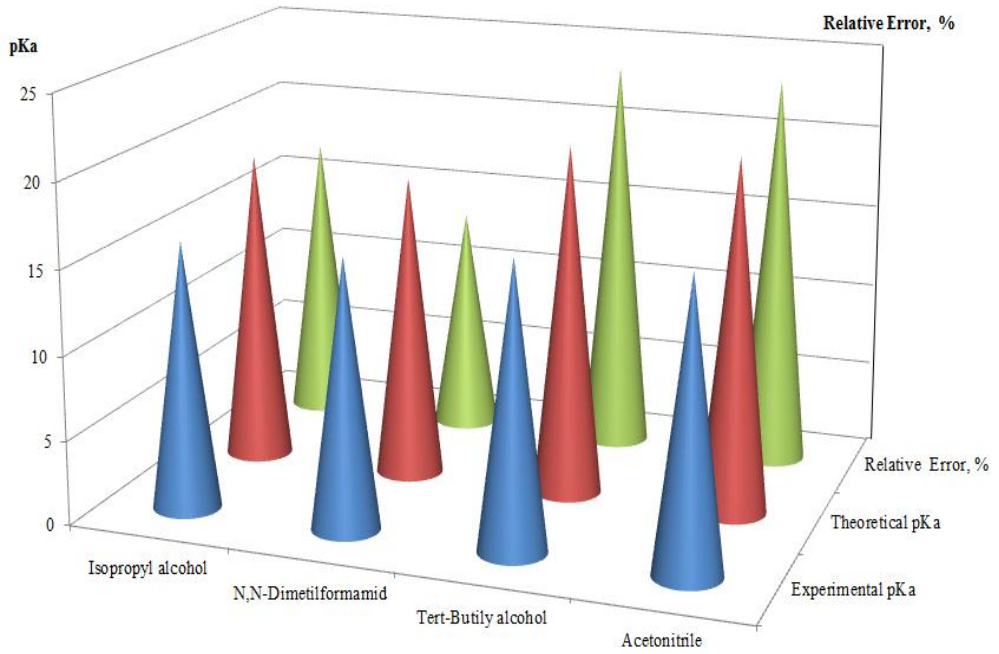


Fig. 5: Experimental and theoretical results compared for compound 1

When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: *N,N*-dimethylformamide (ε = 36.7) > acetonitrile (ε = 36.0) > isopropyl alcohol (ε = 19.4) > *tert*-butyl alcohol (ε = 12.0). In this studied that it is observed as experimental, isopropyl alcohol > *N,N*-dimethylformamide > *tert*-butyl alcohol > acetonitrile for compound **1, 2, 4** and **11**, *N,N*-dimethylformamide > isopropyl alcohol > acetonitrile > *tert*-butyl alcohol for compound **8** and **9**, isopropyl alcohol > *tert*-butyl alcohol > *N,N*-dimethylformamide > acetonitrile for compound **5** and **10**, *N,N*-dimethylformamide > acetonitrile > isopropyl alcohol > *tert*-butyl alcohol for compound **3** and **10**, isopropyl alcohol > acetonitrile > *N,N*-dimethylformamide > *tert*-butyl alcohol for compound **6**, *tert*-butyl alcohol > *N,N*-dimethylformamide > acetonitrile > isopropyl alcohol for compound **7**. But, it is observed as theoretical, *N,N*-dimethylformamide > isopropyl alcohol > *tert*-butyl alcohol > acetonitrile for all compounds.

When analyzed according to autoprotolysis constant, weak acidic property is showed in isopropyl alcohol (pKs: 20.6), *N,N*-dimethylformamide (pKs: 18.0), *tert*-butyl alcohol (pKs: 22.0) and acetonitrile (pKs: 33.0) for all compounds as results of experimental and theoretical.[12] By the time analyzed according to the functional group (-R) effect, it has showed very small effect for acidic protons due to the distance. By the time compounds were analyzed by each solvent, acidity strength decrease; **12** > **11** > **4** > **5** > **8** > **2** > **1** > **10** > **6** > **7** > **9** > **3** in isopropyl alcohol, **12** > **8** > **11** > **10** > **7** > **9** > **2** > **1** > **3** > **5** > **4** > **6** in *N,N*-dimethylformamide, **12** > **11** > **7** > **8** > **5** > **10** > **1** > **2** > **6** > **4** > **9** > **3** in *tert*-butyl alcohol, **8** > **12** > **7** > **10** > **6** > **3** > **9** > **11** > **2** > **1** > **5** > **4** in acetonitrile as observed for experimental, **12** > **8** > **11** > **10** > **7** > **9** > **2** > **1** > **3** > **5** > **4** > **6** in isopropyl alcohol and *N,N*-dimethylformamide, **2** > **12** > **11** > **10** > **9** > **6** > **7** > **8** > **3** > **4** > **5** > **1** in *tert*-butyl alcohol and acetonitrile as observed for theoretical.

Differentiated all compounds showed in the studied solvents when investigated the effect of the leveling and differentiated. When compared theoretical results with potentiometric results was obtained by the half-neutralization method, errors were found to be between -7.01% (compound **6** in *N,N*-dimethylformamide) and -35.00% (compound **12** in *tert*-butyl alcohol).

Antimicrobial

The pyrimidine derivatives were tested for antimicrobial activity against 8 bacteria and 2 yeast. According to the results, it is obtained compound **4** had weak anti-fungal activity but other compounds did not have anti-fungal activity. The results are given in Table 2 and 3. Compounds without antimicrobial activity not given in Table 2. Compound **1**, **2**, **3**, **4**, **5** and **6** were found to be effective against M. *smegmatis* (MIC values between 62.5 and 250 µg) among the tested substances. These substances were also found to be moderately effective against B. *cereus* and S. *aureus* which were Gram-positive sporulated bacilli (MIC values between 62.5 and 125 µg).

Table 2: Antimicrobial activity values of the pyrimidine derivatives

Molecule	Microorganisms and inhibition diameters									
	Ec*.	Bc*.	Ef*.	Pa*.	Yp*.	Sa*.	Lm*.	Ms*.	Sc*.	Ca*.
1	-	10	-	-	-	12	-	>25	-	-
2	-	7	8	-	-	12	10	>25	-	-
3	-	6	-	-	-	12	-	>25	-	-
4	-	7	-	-	-	10	-	>25	-	-
5	-	10	-	-	-	14	-	>25	10	11
6	-	-	-	-	-	-	-	>25	-	-
7	-	6	10	-	-	10	8	12	-	-
8	-	6	-	-	-	9	-	20	-	-
9	-	-	-	-	-	-	-	15	-	-
10	-	-	-	-	-	-	-	16	-	-
11	-	-	-	-	-	10	-	20	-	-
12	-	-	-	-	-	-	-	15	-	-
Amp*	10	15	10	18	18	35	10	-	-	-
Strep*	-	-	-	-	-	-	-	35	-	-
Flu*	-	-	-	-	-	-	-	-	25	25

*Ec: *Escherichia coli* ATCC 25922, Bc: *Bacillus cereus* 702 Roma, Ef: *Enterococcus faecalis* ATCC 29212, Pa: *Pseudomonas aeruginosa* ATCC 43288, Yp: *Yersinia pseudotuberculosis* ATCC 911, Sa: *Staphylococcus aureus* ATCC 25923, Lm: *Listeria monocytogenes* ATCC 43251, Ms: *Mycobacterium smegmatis* ATCC 607 were used as bacteria and strain Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, Ms: *Mycobacterium smegmatis* ATCC607, Amp: Ampicillin (10 µg/mL), Strep: Streptomisin (10 µg/mL), Flu: Fluconazole (5 µg/mL)

Table 3: MIC values of antimicrobial active pyrimidine derivatives

Molecule	Bc.	Lm.	Sa.	Ms.	Ca.	Sc.
1	125	-	125	125	-	-
2	-	250	125	125	-	-
3	-	-	62.5	125	-	-
4	-	-	62.5	125	-	-
5	125	-	62.5	125	125	125
6	-	-	62.5	-	-	-
7	-	-	62.5	125	-	-
8	-	-	125	125	-	-
9	-	-	-	250	-	-
10	-	-	-	250	-	-
11	-	-	-	250	-	-
12	-	-	-	250	-	-
Amp	15	10	35	-	-	-
Strep	-	-	-	4	-	-
Flu	-	-	-	-	<8	<8

CONCLUSION

In this study, ionization constants were studied in four different solvents. This work can be further extended. The number of solvents can be increased and solvents can be selected from different solvent groups. However, the results can be compared using different methods. Biological studies are also more extensible. Different methods are comparable. This way the work becomes more enriched.

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