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Potentiometric Determination of pKa of Some Selected Antibiotics in Mixed Solvent Media

***Eugene-Osoikhia, T. T. and Emesiani, M. C.**
Department of Chemistry, University of Ibadan, Ibadan, Nigeria.
***Correspondence Email:** kemitunmise@yahoo.co.uk

ABSTRACT

The ionization constants of some selected antibiotics namely sulfamethoxazole, trimethoprim and metronidazole have been determined by potentiometry in some mixed solvent media by varying the concentrations at 25ºC. The ionization constants varied with the proportion and nature of different solvent mixtures used, as most exhibited their highest pKa values in 100% DMSO. Specifically, it has been found that in MeOH-DMSO mixture as the proportion of methanol increases, there is reduction in the pKa values for all the antibiotics. In all the solvent mixtures, the pKa values of 7.92, 8.29, 7.61, 7.98, 7.78 (trimethoprim) and 2.99, 2.87, 2.58 and 2.97 (metronidazole) obtained are higher than the reported values at physiological pH for trimethoprim and metronidazole. However, the pKa values obtained for sulfamethoxazole are comparable to the equilibrium dissociation of its amine moiety.

Keywords: Antibiotics, pKa values, mixed solvent, drug permeability, ionization constants

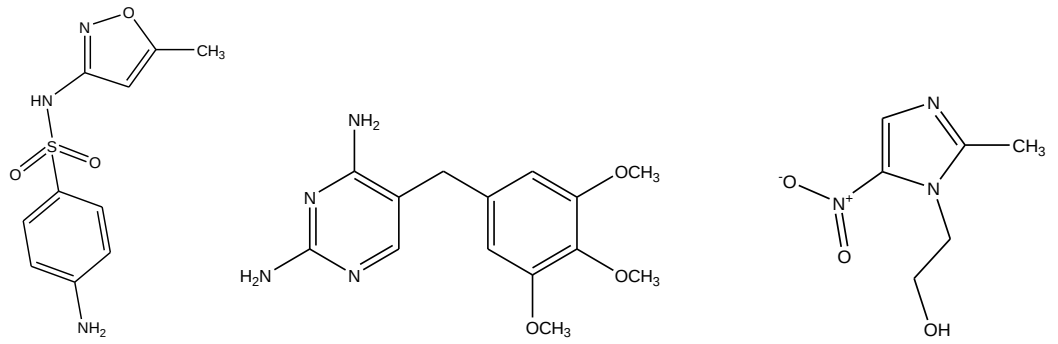
INTRODUCTION

Antibiotics are pharmaceuticals that are commonly used in human and veterinary medicines to prevent or treat bacterial infections. Due to their therapeutic as well as the prophylactic qualities to humans and animals, antibiotics have received widespread use all around the world (Carlson and Fangman, 2000). In industrial pharmacy, the most important physicochemical property of biologically active molecules is their acidity or basicity expressed in terms of pKa values (Sanli *et al*., 2009). Majority of drugs are weak acids and/or bases, hence knowledge of their dissociation constant (pKa) in each case helps in understanding the ionic form a molecule will take across a range of pH values (Manallack, 2007). This is particularly important in physiological systems where ionization state will affect the rate at which the compound is able to diffuse across membranes and obstacles such as the blood-brain barrier (BBB). The pKa of a drug influences lipophilicity, solubility, protein binding and permeability which in turn directly affects pharmacokinetic (PK) characteristics such as absorption, distribution, metabolism and excretion (Settimo *et al*., 2014; Anderson, 2005; Kerns and Di, 2004; Avdeef, 2001).

Solvent molecules are involved in acid-base equilibrium and this makes the strength of an acid dependent on the nature of the solvent. It is well recognized that an ideal solvent (or co solvent) for thermodynamic studies of proton-transfer reactions should have a sufficiently high dielectric constant to promote dissolution of ionic compounds or dissociation of ionizable compounds, a low autoprotolysis constant, and a low leveling power. Consequently, amphiprotic solvents (e.g., alcohols) which include either protogenic (acidic) or protophylic (basic) solvents have been widely used for this purpose (Bretti *et al*., 2013; Subirats *et al*., 2007; Kutt *et al*., 2006; Kaljurand *et al*., 2003;). The poor aqueous solubility of many weak electrolyte drugs has necessitated the use of water-miscible co solvents to prepare solutions of these compounds for potentiometric determination of the pKa (Jouyban et al., 2002). On the other hand, mixed solvents are often employed to obtain correlations at different solvent compositions. Although dissociation constants of acids and bases in mixed and non-aqueous solvents have been extensively investigated (Ahmed *et al*., 2004) little work has been done to study the effect of varying the concentrations of the mixed solvents on the pKa of drugs.

As many drugs have the potential to exist as ionic species when dissolved in a variety of biological matrices, it has been reported that most drugs are ionized in the range of 60−90% at physiological pH (Manallack *et al*., 2013). On this basis, the pKa of some selected antibiotics (Fig. 1) sulfamethoxazole, trimethoprim, and metronidazole was measured by potentiometric technique by varying the concentrations of the mixed solvents of the organic media: amphiprotic (methanol and ethanol) and dipolar aprotic dimethyl sulfoxide (DMSO) in the range 50% to 100%. The pKa

values were observed in relation to both the proportion and nature of the organic solvent.



(a) (b) (c)

Figure 1: Structures of selected antibiotics (a) sulfamethoxazole (b) trimethoprim (c) metronidazole

Sulfamethoxazole is a synthetic antibacterial drug belonging to the sulfonamides, used in the treatment of urinary tract infections (Tripathi *et al*., 2012; Suganya and Kabilan, 2004; Haasnoot *et al*., 2000; Owa, and Nagasu, 2000;). Sanli *et al* (2009) reported that sulfonamides such as sulfamethoxazole contains two important functional groups in the pharmaceutically relevant pH range of 4 to 9; one acidic amide moiety and one basic amine moiety. Trimethroprim (pKa=7.2) is an antifolate drug (Teresa *et al*., 2011). It selectively inhibits the bacterial form of the dihydrofolatereductase enzyme (Bekçi *et al*., 2006; Batt and Aga, 2005; Diaz-Cruz *et al.*, 2003; Raj *et al*., 2003). It had been reported that the pKa values of sulfamethoxazole and trimethroprim determined by potentiometric method in acetonitrile-water mixture at ambient tempearature were 1.85 and 5.60 respectively (Sanli *et al*., 2009). Metronidazole is an antibacterial agent that was found to exhibits a wide range of biological activities ranging from antibacterial and antiparasitic to anticancer (Saadeh., 2009). Moreover, metronidazole has been proposed as a drug of choice against *Helicobacter pylori*, which is considered the etiological agent for active chronic gastritis (Ayla and Nurcan, 2006).

METHODOLOGY

General: Orion model 701A digital pH-meter (accurate to pH = + 0.005 units) with a glass calomel electrode assembly at constant temperature (25.00 ± 0.10°C) was used to determine the pKa values**. S**ulfamethoxazole, trimethroprim and metronidazole, antibiotics were gifts from Unique Pharmaceutical, Ogun State and Bond Chemicals Industry, Awe, Oyo State. Nigeria. Sodium hydroxide, Potassium Hydrogen phthalate (KHP), Hydrochloric acid, Ethanol, Methanol, Dimethylsulfoxide (DMSO) were of analytical grade obtained from Sigma Aldrich and used without further purification. The stock solutions of the compounds were prepared by dissolving the appropriate amount of each substance in redistilled water. CO_2-free NaOH solution (0.2 $moldm^{-3}$) was prepared and standardized against a standard solution of potassium hydrogen phthalate. More dilute solutions were prepared by accurate dilution.

Procedure. Following a standard procedure the pH meter was calibrated with buffer solutions pH 4.0, pH 7.0 and pH 10.0 prior to use. The titrant 0.1M NaOH was standardised using 0.1M KHP (potassium hydrogen phthalate). Concentrations of the antibiotics samples 0.01M were prepared in different solvent mixtures 50% Ethanol-water mixture, 50% Ethanol- Methanol mixture, 75% Ethanol- Methanol mixture, 50% Methanol-DMSO mixture, 75%Methanol-DMSO mixture and 50% Methanol- water mixture, 100% ethanol, 100% methanol and 100% DMSO.

Antibiotic sample (40 cm^3) was pipette into a 100 cm^3 beaker followed by the addition of two drops of 1M HCl solution. The solution was placed in a magnetic stirrer and stirred continuously with the pH electrode dipped in it. The pH of the solution was taken at volume zero of the titrant NaOH. This was followed by repeated addition of 0.2 cm^3 aliquots of 0.1M NaOH at each time and the corresponding pH values taken after each stirring. The equivalence point was soon observed and the titration continued until further addition of the titrant produced a constant pH value. A plot of pH against volume of titrant was carried out. From the plotted graph, the experimental pKa value was deduced. The volume of NaOH required at the equivalence point was extrapolated from the graph and the pH at half this volume gave the experimental pKa. Results were

obtained in duplicates for all the samples studied. (Qiang and Adams, 2004)

RESULTS AND DISCUSSION

The experimental pKa values obtained for the antibiotics sulfamethoxazole, trimethoprim and metronidazole in their solvents mixtures are presented in Tables 1-3. The graphical plots are shown in Figures 2-4 respectively. The obtained pKa values are discussed in terms of both the proportion and nature of the organic solvents.

Table 1: Summary of the pKa values of Sulfamethoxazole in solvent mixtures at 25ºC

Compound	100% DMSO	75% MeOH-DMSO	50% MeOH-DMSO	100% MeOH	75% EtOH-MeOH	50% EtOH-MeOH	100% EtOH	50% EtOH-H_2O
Sulfamethoxazole	3.92(4.25±0.2)	2.51(2.52±0.1	2.66(2.92±0.1)	2.15(2.17±0.2	0.86(0.93±0.1	0.75(1.15±0.2	2.78(2.84±0.2	2.79(5.67±0.2

(theoretical value) , MeOH =Methanol, EtOH = Ethanol, DMSO = Dimethylsulfoxide, H_2O = Water

Table 2: Summary of the pKa values of Trimethoprim in solvent mixtures at 25ºC

Compound	100% DMSO	75% MeOH-DMSO	50% MeOH-DMSO	100% MeOH	75% EtOH-MeOH	50% EtOH-MeOH	50% MeOH-H_2O
Trimethroprim	8.41(8.63±0.2)	7.98(7.90±0.3	8.29(8.51±0.2)	7.90(7.94±0.1)	7.78(7.89±0.1	7.92(8.18±0.2)	7.61(7.76±0.1)

(theoretical value) , MeOH=Methanol, EtOH = Ethanol, DMSO = Dimethylsulfoxide, H_2O = Water

Table 3: Summary of the pKa values of Metronidazole in solvent mixtures at 25ºC

Compound	100% DMSO	75% MeOH-DMSO	50% MeOH-DMSO	100% MeOH	75% EtOH-MeOH	50% EtOH-MeOH	100% EtOH	50% EtOH-H_2O
Metronidazole	3.66(4.14±0.3	2.58(2.76±0.1	2.87(3.11±0.1	2.72(2.70±0.1	2.97(3.42±0.3	2.99(3.18±0.1	2.73(2.99±0.1	1.62(1.74±0.1

(theoretical value) , MeOH =Methanol, EtOH = Ethanol, DMSO = Dimethylsulfoxide, H_2O = Water

The pKa values cited in Table 1-3 indicate that the acid ionization constants are largely dependent on both the proportion and the nature of the organic solvent as it varies with proportion and nature of different solvent mixtures used. From the results obtained all the antibiotics used gave their highest pKa values trimethoprim (8.41), sulfamethoxazole (3.92) and metronidazole (3.66) in 100% DMSO a dipolar aprotic solvent due to its high dielectric constant and solvating ability which plays a fundamental role in dissociation reactions (Laurence and Gal, 2010; Izutsu, 2009; Brotzel *et al*., 2007) by stabilizing the produced anion and hydrogen ion, hence increase in pKa.

It was observed that in MeOH-DMSO solvent mixture as the proportion of methanol increases, there is reduction in the pKa values. For instance, trimethoprim gave pKa value of 8.29 in 50% MeOH-DMSO while the value decreases to 7.98 as the proportion of methanol increases to 75% in the solvent mixture, the same trend was observed in sulfamethoxazole and metronidazole in 50% and 75% MeOH-DMSO mixture. This is because DMSO with a higher dielectric constant (ϵ = 47) can dissociate and stabilize the produced anion and hydrogen ion but presence of higher proportion of solvent such as methanol with lower dielectric constant (ϵ = 32.6), decreases the extent of interaction between the acid anion and proton with solvent. Hence, this decreases the acidity constants of the sample, and the eventual reduction in pKa values at 75% proportion.

Generally, the observed pKa values in 100% ethanol and methanol (amphiprotic solvents) Table1-3 were comparable except for sulfamethoxazole where the pKa value in 100% ethanol (2.78) was far greater than the observed value of 2.15 in 100% methanol is in consistent with the fact that ethanol is characterized by a lower tendency to donate hydrogen bond than methanol(with increasing the molecular weight of alcohol (Dixit *et al*, 2002). This was deduced from the fact that the tendency of alcohol to associate with solutes through hydrogen bond decreases with increasing the molecular weight of alcohol (Synder *et al*., 2011; Ahmed *et al*., 2004; Dixit *et al*., 2002; Boraei et al., 2001). These concepts suggested that the hydrogen bond donating power of the solvent has a conspicuous effect on the acid ionization for compounds under investigation.

For Trimethoprim , all the exhibited pKa values are higher than the reported value of 7.4 in physiological medium(Dalhoff *et al*., 2011). This may be attributed to the contributing effects of factors such as hydrogen bonding, solvent basicity, dispersion forces as well as proton-solvent interaction which might have profound influence on the ionization process. For instance, the pKa of 7.61 obtained in 50% MeOH-H_2O mixture can be explained in terms of the lower hydrogen bonding

donating ability of methanol as well as its less basic character which leads to a lower degree of ionization of the trimethoprim. This gives a high pKa values when compare with the pure aqueous medium.

Da Silva *et al*., 2017 reported that trimethoprim showed three ionized forms and one neutral form, and that the ionized form predominate in solution with pH 4.3-5.8 i.e slightly acidic pH . However, the solubility drops as the pH increases, thus, at higher pKa value , like in 100% DMSO, trimethoprim remained largely non-ionized (only 2.5% ionized), thus, in all the proportion of mixed solvents medium used, trimethoprim exist in unionized form which will facilitate its penetration through the cell membrane of the microbial cell (Ungheri *et al*., 2002), thus inhibiting growth. By contrast, at pH 5, 99.6% of trimethoprim was ionized, which reduced its ability to penetrate the cell membrane (AlRabiah *et al*., 2018).

Metronidazole is a weak base with a pKa of 2.52 (Putra *et al*., 2009). The pKa values obtained in other mixed solvents are higher in value to the reported value for metronidazole except for the values in 50% EtOH-H_2O of 1.62, which is more acidic. In 75% MeOH-DMSO, it exhibited pKa value of 2.58 which is comparable to the reported value of 2.52 in physiological medium. It was reported that metronidazole is predominantly unionized at physiological pH (pKa 7.4), hence in the blood it exist as unionized form but in the stomach (pKa 1.5 -3.5) it is in ionized form (Putra *et al*., 2009). Thus, it can be inferred that in all the mixed solvents used except in 100% DMSO, metronidazole was present in ionized form. However, in 100% DMSO, it was in unionized form which is more lipophilic and can penetrate through cell membrane.

Sulfamethoxazole,a sulfonamide contains two important functional groups in the pharmaceutically relevant pH range of 4 to 9, one acidic amide moiety and one basic amine moiety. The amine nitrogen atom (-NH_2) is able to gain a proton, while the amide nitrogen atom of the amide group is able to release a proton under specific pH conditions. Thus, the first dissociation equilibrium refers to the dissociation equilibrium of the amine moiety (pK1) and the second equilibrium to the dissociation equilibrium of the amide moiety (pK2). Reported values for pK1 due to amine moiety in water by potentiometry are 2.06±0.30 and 1.85±0.30 and for pK2 due to amide moiety are 6.90±0.05 and 5.60±0.04 respectively (Garcia-Campana et al., 2009; Barbic *et al*., 2007; Day *et al*., 2007; Volgyi, 2007; Qiang and Adams, 2004). Comparing these values in water with the values obtained in the range 3.92 to 0.75 in 100% DMSO and EtOH-H_2O mixed solvents, the exhibited dissociation equilibrium was only due to pK1 that dissociation equilibrium of amine moiety, which invariably suggest that the amide moiety did partake in equilibrium dissociation in the mixed solvent used.

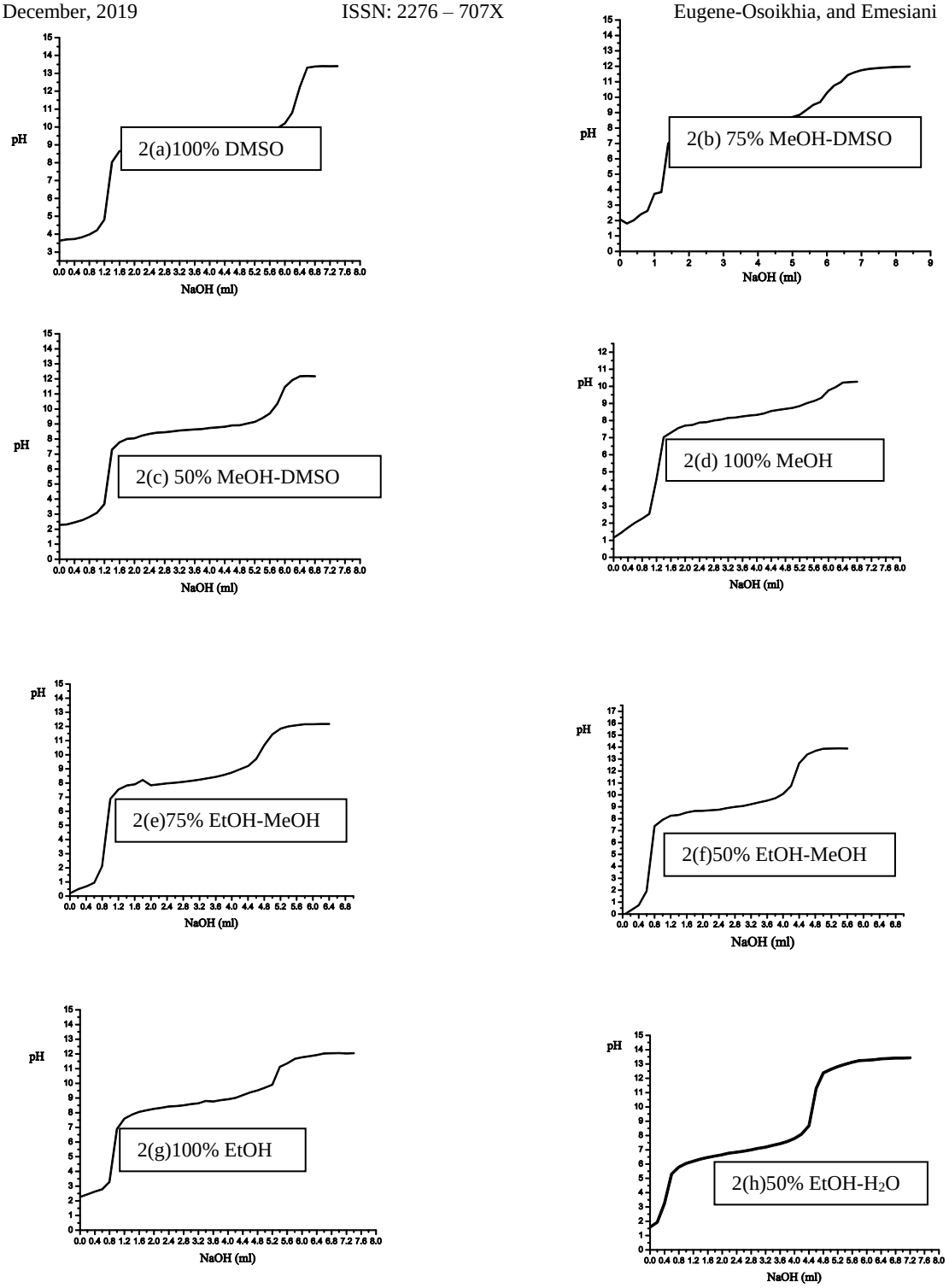


Figure 2: Titration curves of Sulfamethoxazole in different solvent mixtures at 25°C

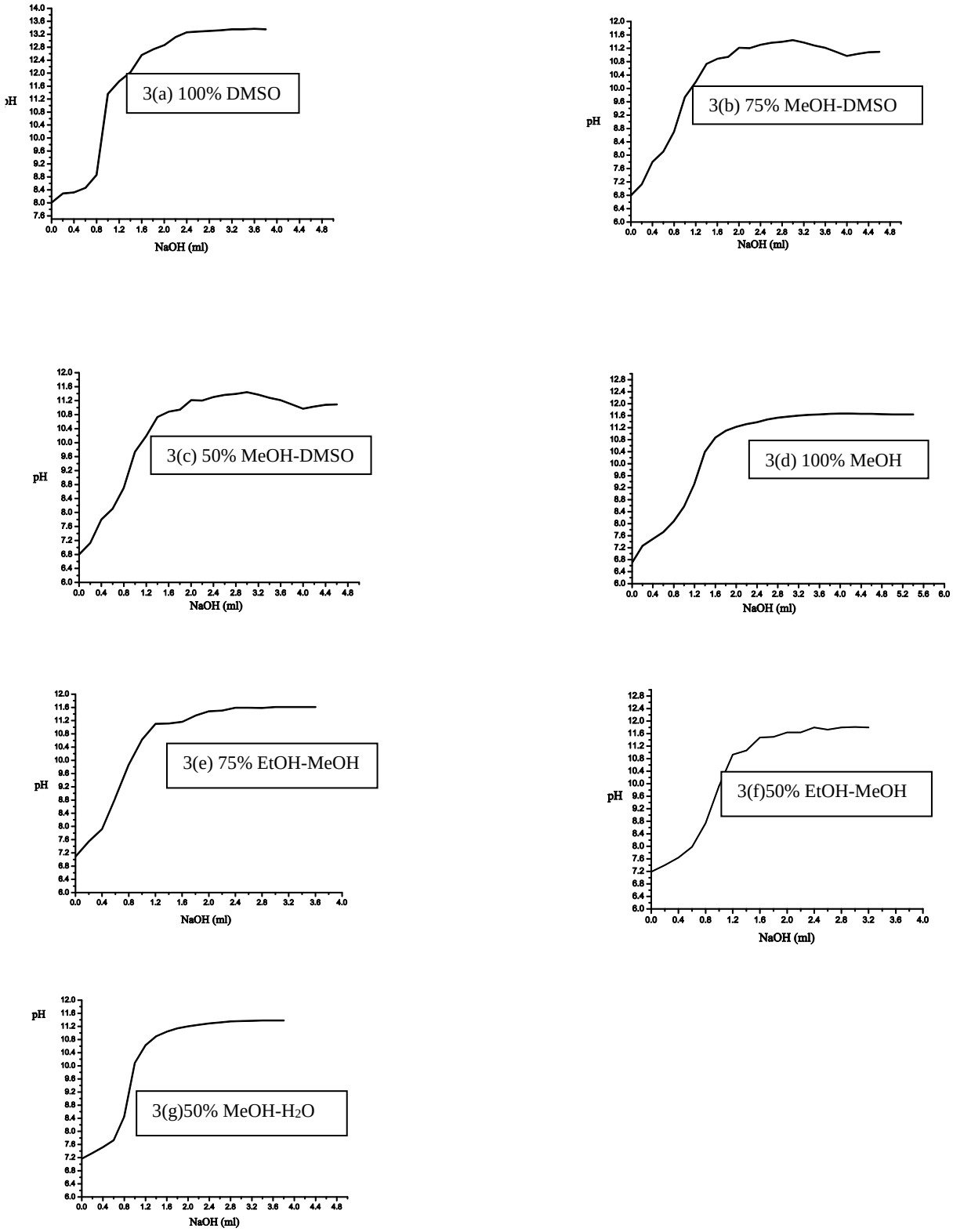


Figure 3: Titration curves of Trimethoprim in different solvent mixtures at 25°C

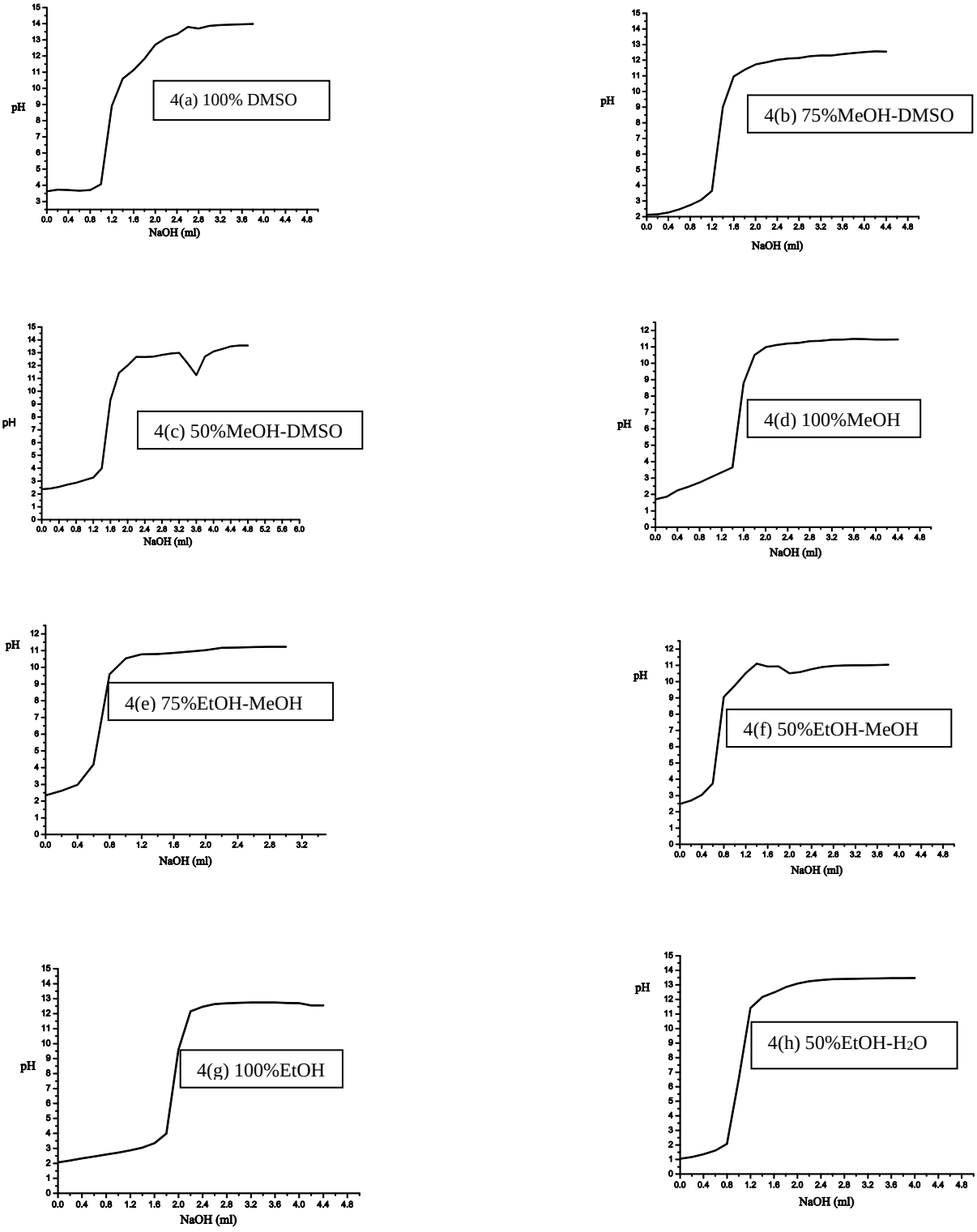


Figure 4: Titration curves of Metronidazole in different solvent mixtures at 25°C

CONCLUSION

The ionization constants of some selected antibiotics determined varied with the proportion and nature of different solvent mixtures used. The highest pKa values of all the antibiotics was obtained in 100% DMSO. In MeOH-DMSO solvent mixture as the proportion of methanol increases, there is reduction in the pKa values for all the antibiotics. In all the solvent mixtures, the pKa values obtained are higher than the reported values at the physiological pH for trimethoprim and metronidazole. However, the pKa values obtained for sulfamethoxazole are comparable to the equilibrium dissociation of its amine moiety.

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