SPECTROFLUORIMETRIC DETERMINATION OF TETRACYCLINE AND TERBUTALINE SULPHATE IN ITS PURE AND DOSAGE FORMS USING EOSIN Y REAGENT

Theia'a N. Al-Sabha[a]*, Mohamed Y. Dhamra[a] and Thabit S. Al-Ghabsha[b]

Keywords: Tetracycline; terbutaline sulphate; Eosin Y; fluorimetry.

A spectrofluorimetric method has been developed for the determination of tetracycline hydrochloride and terbutaline sulphate in different dosage forms. The method is based on the quantitative quenching effect of tetracycline hydrochloride and terbutaline sulphate on the native fluorescence of Eosin Y at the pH 6.4 and 3.5 respectively. The quenching of the fluorescence of Eosin Y was measured at 545 nm after excitation at 350 nm. The fluorescence-concentration plots are rectilinear over the range 0.5–18 and 0.05-5.0 μg mL^{-1} with LOD of 0.531 and 0.241 μg mL^{-1} and LOQ of 1.77 and 0.806 μg mL^{-1} for above drugs respectively. The proposed method has been successfully applied to the analysis of commercial tablets and capsules containing the drug. Statistical comparison of the results with those of the reference method revealed good agreement and proved that there were no significant differences in the accuracy and precision between the two methods.

* Corresponding Authors
E-Mail: dr_theiaa@yahoo.co.uk
[a] Chemistry department, College of Education, Mosul University, Mosul, Iraq
[b] College of Pharmacy, Mosul University, Mosul, Iraq

INTRODUCTION

Terbutaline sulphate [2-(*tert*-butylamino)-1-(3,5-dihydroxyphenyl)ethanol sulphate][1] is a short-acting bronchorelaxant which can be given orally,[2] it is readily metabolized in the gut wall and liver when given orally. It has a short duration of action.[3] It has the following chemical structure (Figure 1).

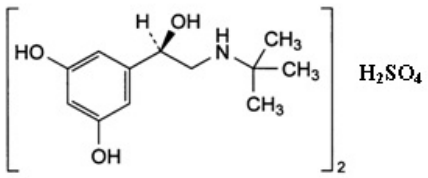

Figure 1. Structure of terbutaline sulphate.

Terbutaline sulphate is widely used as an effective bronco dilator in the management of asthma. This is used as prophylactic drug as well as to prevent acute exacerbations of asthma, chronic bronchitis, emphysema and other lung diseases. It relaxes and opens air passage in the lungs, making it easier to breathe[3,4].

Tetracycline hydrochloride [(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12 apentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxa-mide hydrochloride][1] is an effective antibiotic in treating infections,[5] and its absorption is reduced by anti-acids and milk, because it can form insoluble complexes with Ca, Mg, Al and Fe.[6] It has the following chemical structure (Figure 2).

Tetracycline is an antibiotic with a broad antibacterial spectrum and bacteriostatic activity, having a good activity against acute disease caused by gram-positive and gram-negative bacteria, including the species like *Spirochete, Actinomyces, Ricketsia* and *Mycoplasma*.[7]

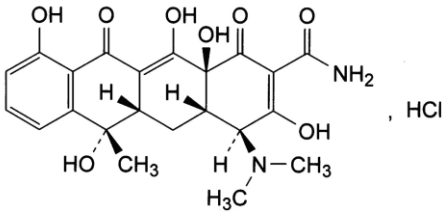

Figure 2. Structure of tetracycline hydrochloride.

Different analytical techniques have been developed for determination of terbutaline sulfate and tetracycline. HPLC,[8,9] LC–MS,[10] CE,[11] CE–MS,[12] voltammetry,[13] electrochemical method,[14,15] liquid chromatography,[16,17] capillary electrophoresis[18] and chemiluminescence[19,20] have been reported for determination of tetracycline. These methods are often time-consuming, expensive, and cumbersome.

Spectrophotometric methods have been reported for determination of both drugs using different reagents.[21-30] Spectrofluorimetry is attractive because of its sensitivity, speed, and simplicity. Most of the additives or excipients found in pharmaceutical preparations are not fluorescent in nature. The aim of this study is to develop an optimized spectrofluorimetric method for the determination of tetracycline hydrochloride and terbutaline sulphate in pharmaceutical formulations. The present spectrofluorimetric method is based on the formation of ion pair complex of tetracycline hydrochloride and terbutaline sulphate with Eosin Y at the pH 6.0 and 3.55 (sodium acetate–acetic acid buffer solution) respectively.

Experimental

Fluorescence spectral measurements were made on a RF-5301PC-Spectrofluorophotometer (Tokyo, Japan) equipped with a xenon lamp and 10 mm quartz cells. Excitation and emission wavelengths were set at 350 and 545 nm with the excitation and emission slit widths of 10 nm, respectively. All measurements were performed at 28±1 °C. Philips PW 94 supplied with CE 10-12 pH electrode was used for pH measurement.

All reagents were of analytical-reagent grade which were provided by BDH and Fluka companies. Stock solutions of terbutaline sulphate and tetracycline hydrochloride drugs were prepared in concentration of 50 μg mL^{-1} by dissolving 0.01 g of each in distilled water and made up to 200 mL in volumetric flask. The solutions were kept in refrigerator. The Eosin Y solution of 50 μg mL^{-1} was prepared by dissolving 0.01g in distilled water and made up to 200 mL in a volumetric flask. The *acetate buffer* solution was prepared with pH 6.0 and 3.55 by mixing sodium acetate and acetic acid solutions of 0.1 M and adjusted by pH meter.

General procedure

Aliquots of solution containing 0.1-3.6 mL and 0.01-1.0 mL of 50 μg mL^{-1} for tetracycline hydrochloride and terbutaline sulphate were added separately into two series of 10-mL volumetric flasks containing 2.5 mL and 2.0 mL of sodium acetate–acetic acid buffer solution of pH 6.0 and 3.5 respectively and 3.0 mL of 50 μg mL^{-1} Eosin Y. The solutions were diluted to the mark with distilled water and mixed well. The fluorescence intensity (ΔF) was recorded at 545 nm after excitation at 350 nm. The amount of the drugs was obtained either from their corresponding calibration graphs or the regression equations.

Procedure for pharmaceutical formulations

Tetracycline chloride capsule

Seven tetracycline hydrochloride capsules content (each capsule contains 250 mg tetracycline hydrochloride) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to one capsule was accurately weighed and dissolved in 5 mL ethanol for increasing dissolution and made up to 100 mL with distilled water. The mixture was mixing well and filtered through Whatman no.42 filter paper. The filtrate was diluted to the 250 mL with distilled water to obtain 1000 μg mL^{-1} concentration. A suitable volume was diluted, and the general procedure was followed.

Terbutaline tablet

Twenty terbutaline sulphate tablets (each tablet contains 5 mg terbutaline sulphate) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to one tablet was accurately weighed and dissolved in distilled water, mixing well and filtered through Whatman no.1 filter paper. The filtrate was diluted to the 100 mL with distilled water obtain a concentration of 50 μg mL^{-1}. A suitable volume was diluted, and the general procedure was followed.

Results and Discussion

In the present study, tetracycline hydrochloride and terbutaline sulphate were found to form ion pair red complexes with Eosin Y at pH of 6.0 and 3.55 respectively, with maximum absorbance at 545 nm (Figure 3). The complexes are formed mainly due to the electrostatic interaction between the studied drug and anionic functional group of Eosin Y at suitable pH. The formed ion pair complexes are not fluorescent, therefore, the decrease in the fluorescence intensity of Eosin Y upon the addition of the drugs was the basis for the spectrofluorimetric measurement at 545 nm after excitation at 350 nm (Figure 4a, 4b).

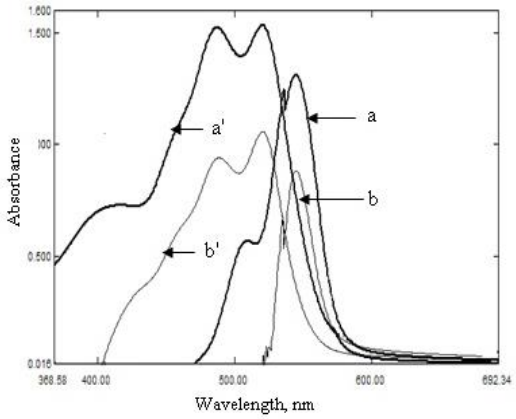

Figure 3. Absorption spectra of (a) 8 μg mL^{-1} terbutaline sulphate and (b) 40 μg mL^{-1} tetracycline hydrochloride with Eosin Y against their respective reagent blank (a', b') under optimum cond···

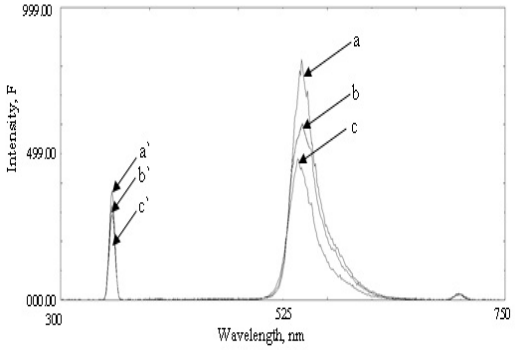

Figure 4a Excitation and emission spectra of: (a`, b`, c`) Blank Eosin Y (15 μg mL^{-1}) at pH 6.4; (a, b, c) Reaction product of Eosin Y (15 μg mL^{-1}) and tetracycline hydrochloride (0.0, 2.0 and 12.0 μg mL^{-1}).

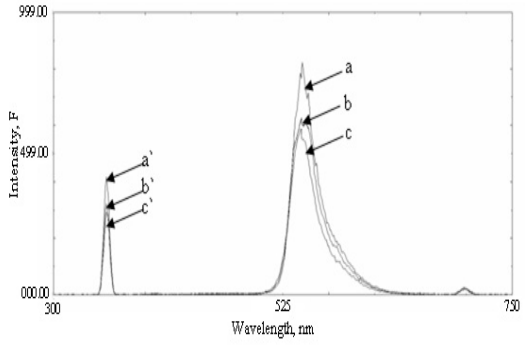

Figure 4b. Excitation and emission spectra of: (a`, b`, c`) Blank Eosin Y (15μg mL^{-1}) at pH 6.4; (a, b, c) Reaction product of Eosin Y (15 μg mL^{-1}) and terbutaline sulphate (0.0, 4.0 and 5.0 μg mL^{-1}).

Optimization of Experimental Parameters

The different experimental parameters affecting the development of the reaction products and its stability were studied and optimized for the spectrofluorimetric method. Such parameters were changed individually while others were kept constant. These parameters include selection of Eosin Y concentration**,** pH, type of buffer and its volume, temperature, reaction time and effect of solvent.

Selection of Eosin Y concentration

To select the concentration of Eosin Y for determination of the drugs, a calibration curve was prepared by addition aliquots of 50 µg mL^{-1} Eosin Y in a set of 10-mL calibrated flasks and diluted to the mark with distilled water. The fluorescence intensity was measured after 5 min. at 545 nm after excitation at 350 nm. Beer's law was obeyed in the range 0.5-15 µg mL^{-1} (Figure 5). However; 15 µg mL^{-1} of Eosin Y was selected in this study.

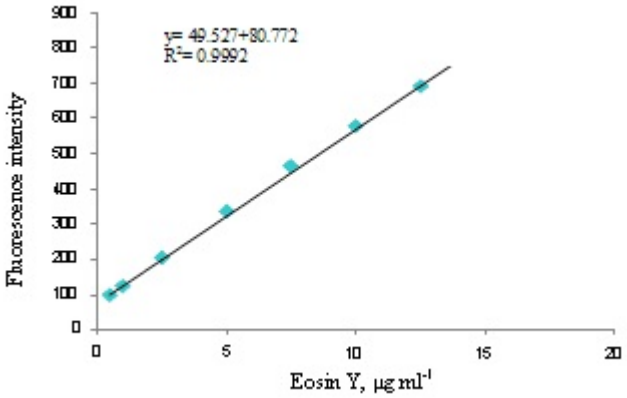

Figure 5. Calibration curve of Eosin Y.

Effect of pH and buffers

When tetracycline hydrochloride added to the Eosin Y, a quenching of the fluorescence intensity for Eosin Y was immediately observed. The final pH of the solution was measured and found 6.0. But quenching of Eosin Y by addition of terbutaline sulphate was observed in the presence of acid with pH 3.55 at final dilution. Therefore, different buffers as phthalate, acetate and citrate of pH 6 and 3.55 were prepared to obtain high ΔF for above drugs respectively. However; acetate buffer was gave maximum ΔF for both drugs and chosen as the optimum throughout the study. It was found that 2.5 and 2.0 mL of acetate buffer gave high ΔF for above drugs respectively (Figure 6).

Effect of heating time and temperature

The effect of temperature, at room temperature (27 °C) and 40 °C, and of time on the quenching of the fluorescence intensity of Eosin Y was studied. It was found that the decrease in the fluorescence intensity of Eosin Y was immediate upon addition of drug in the presence of acetate buffer solution at room temperature and remained constant for more than 90 min for tetracycline hydrochloride and more than 120 min for terbutaline sulphate (Figure 7). However, standing times of 5 and 10 min at room temperature were chosen for tetracycline hydrochloride and terbutaline sulphate, respectively.

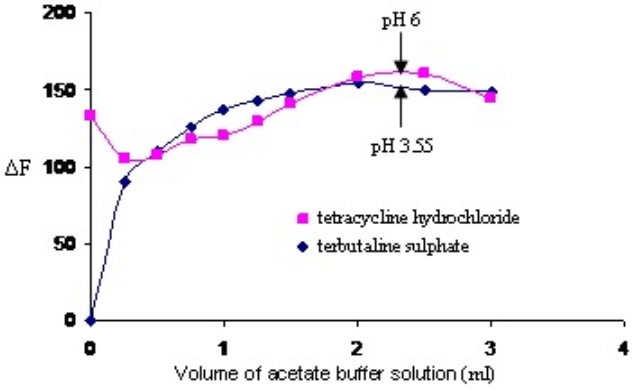

Figure 6. Effect of the volume of acetate buffer solution on ΔF of the tetracycline hydrochloride (2.5 µg mL^{-1}) and terbutaline sulphate (2.5 µg mL^{-1}) with 15 µg mL^{-1} Eosin Y.

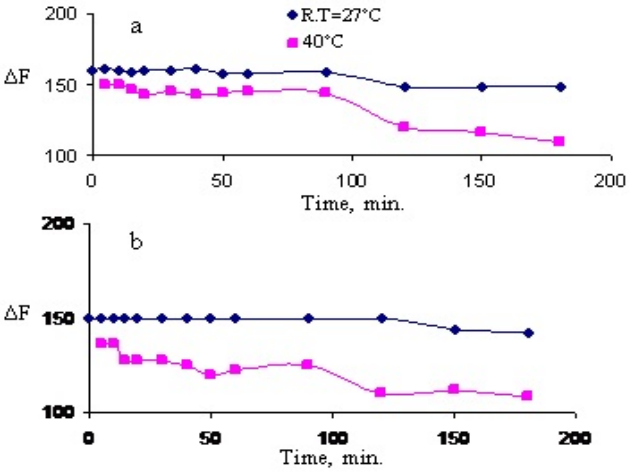

Figure 7. Effect of temperature and standing time on the fluorescence intensity (ΔF) for 2.5 µg mL^{-1} of (a) tetracycline hydrochloride and (b) terbutaline sulphate ion pair complexes with 15 µg mL^{-1} Eosin Y.

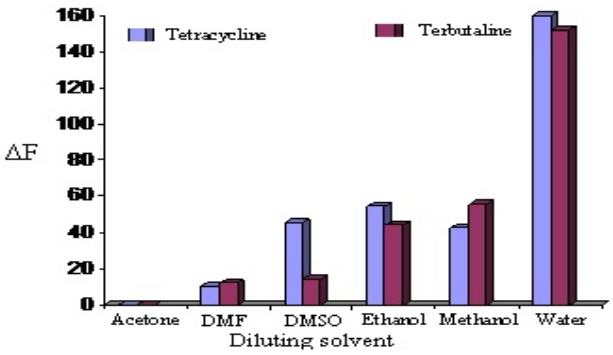

Figure 8. Effect of organic solvent and water on the fluorescence intensity (ΔF) for 2.5 µg mL^{-1} of tetracycline hydrochloride and terbutaline sulphate ion pair complexes with 15 µg mL^{-1} Eosin Y.

Effect of diluting solvents

Dilution effect with different organic solvents, such as acetone, DMF, DMSO, ethanol and methanol in addition to water, were tested on the relative fluorescence intensity. The results revealed that best solvent was water, where as the organic solvents diminished the fluorescence of Eosin Y (Figure 8). Therefore, water was used as diluting solvent.

Effect of surfactant

In order to improve fluorescence intensity (ΔF), various surfactants such as sodium dodecyl sulphate (SDS), cetyltrimethyl ammonium bromide (CTAB), Tween 80 (Tw-80), Triton X-100 (Tr-100), and cetylpyridinium chloride (CPC) were added to the tetracycline hydrochloride and terbutaline sulphate solutions, and their effect was studied. None of the studied surfactants had significant effect on ΔF for both drugs (Figure 9).

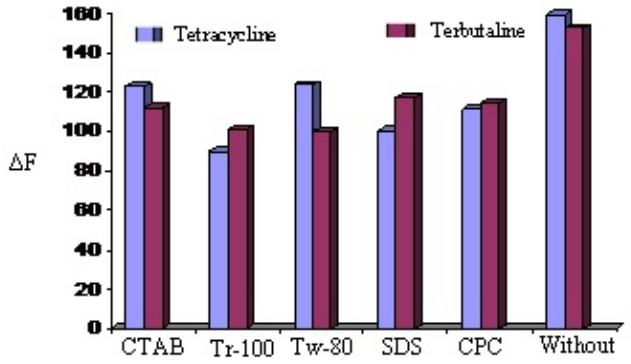

Figure 9. Effect of surfactants on the fluorescence intensity.

Effect of order of addition of reagents

Series of solutions were prepared with different orders of addition of reagents but the same concentrations of reagents, and their corresponding blank solutions were measured at λex/λem = 350 nm/545 nm. The results shown in Figure 10 indicate that addition of acetate buffer followed by addition of Eosin Y and drug gave maximum ΔF and was used in general procedure.

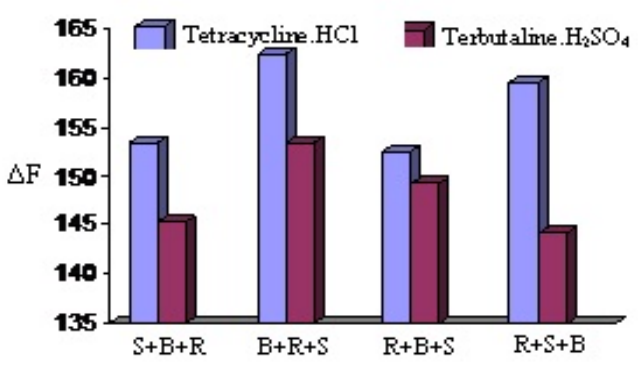

Figure 10. Effect of addition order on the ΔF for tetracycline.HCl (2.5 μg mL^{-1}) and terbutaline.H_2SO_4(2.5 μg mL^{-1}) whereas *S*= drug, *B*=buffer solution and *R*= Eosin Y.

Selectivity

The selectivity of the proposed method was evaluated by analyzing the standard solutions of tetracycline hydrochloride and terbutaline sulphate in the presence of some excipients such as cited in table 1. It was observed that these excipients did not interfere with the proposed method. The results of the recovery experiment also indicated that accuracy is not affected by the co-formulated substances.

Method validation

Linearity, limits of detection and quantitation

Under the optimized experimental conditions, the calibration graphs were constructed by plotting the difference in fluorescence intensity (ΔF) as a function of the corresponding tetracycline hydrochloride and terbutaline sulphate concentrations in μg mL^{-1} (Figure 11). The linear relationships were obtained in the concentration range 0.5–18 and 0.05-5.0 μg mL^{-1} for above drugs respectively. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following formulae

$$LOD = 3.3\sigma/b \text{ and } LOQ = 10\sigma/b$$

where σ is the standard deviation of five reagent blank determinations and *b* is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range (Table 2).

Accuracy and precision

The accuracy was checked by five times analysis for three different concentrations of pure samples. The results obtained in Table 3 showed the close agreement between the measured and true values indicating good accuracy of the proposed method. The calculated relative standard deviation (RSD) values were found to be ≤ 2.58 % for tetracycline hydrochloride and ≤ 3.87 % for terbutaline sulphate indicating good repeatability and reliability of the proposed methods (Table 3).

Method validation and applications

To evaluate the analytical applicability of the proposed method, it was successfully applied to determine tetracycline hydrochloride and terbutaline sulphate in some pharmaceutical preparations. The obtained recovery % values cited in Table 4 indicated high accuracy and there is no serious interference in the determination of above drugs in such samples. The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method (Table 4), by applying the F-test and the t-test at 95% confidence level with five degrees of freedom. The calculated values for F and t tests for proposed method did not exceed the theoretical values (F = 5.05, t = 2.571). This confirmed that there are no significant differences between the proposed method with BP method for tetracycline HCl and terbutaline sulphate.

Table 1. Effect of excipients for assay of tetracycline. HCl and terbutaline. H_2SO_4.

Excipient	Recovery % of 2.5 µg mL^{-1} tetracycline.HCl [Exciepient] µg mL^{-1}			Recovery % of 2.5 µg mL^{-1}terbutaline.H_2SO_4 [Exciepient] µg mL^{-1}		
	100	500	1000	100	500	1000
Starch	97.34	96.65	95.54	95.92	95.54	95.43
Glucose	100.39	97.75	95.90	98.97	97.93	95.69
Lactose	98.95	98.00	97.20	97.92	98.34	98.44
Sucrose	95.53	96.39	97.24	97.72	98.23	97.41
KCl	102.35	103.21	103.25	98.99	99.73	98.54
NaCl	98.78	99.85	97.39	100.89	102.00	99.99
Na_2SO_4	94.23	94.54	95.02	95.00	95.09	95.85
Mg-stearate	98.54	97.07	97.32	99.21	99.39	99.91

Stoichiometry

The stoichiometric ratio between tetracycline hydrochloride and Eosin Y at pH 6.4 and terbutaline sulphate and Eosin Y at pH 3.55 were evaluated by mole ratio method[32] according to following equation: A_{max} = f ([Eosin Y] / [drug]), Where the concentration of drugs and Eosin Y are identical (1.04×10^{-4}M for tetracycline hydrochloride and 9.11×10^{-5}M for terbutaline sulphate). By the change of Eosin Y volume and keeping the volume of drug constant (0.5 mL) in final volume of 10 mL, the procedures were completed using the optimum conditions for each drug. The results confirm that the ratio of complexes Eosin Y : drug are equal to 2:1 (Figure 12).

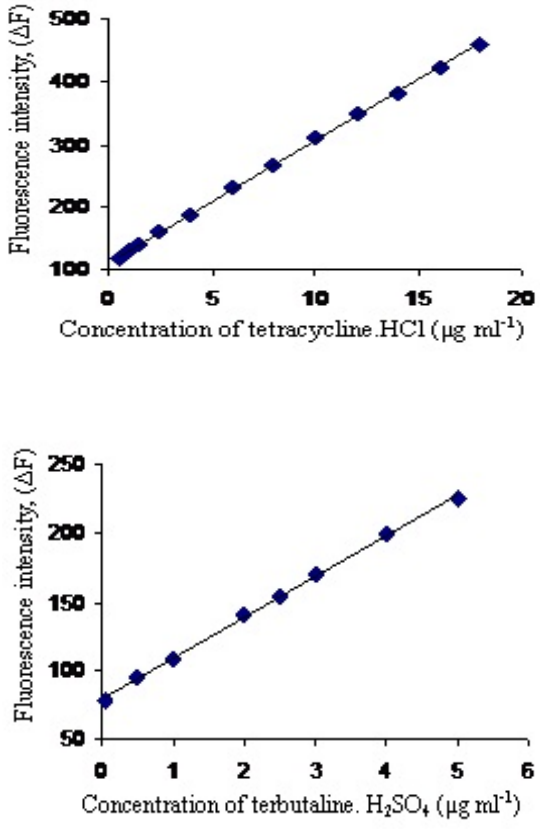

Figure 11. Calibration graphs for determination of tetracycline hydrochloride and terbutaline sulphate

Table 2. Statistical parameters for the determination of tetracycline hydrochloride and terbutaline sulphate.

Parameters	Tetracycline hydrochloride	Terbutaline sulphate
λ_{ex} (nm)	350	350
λ_{em} (nm)	545	545
Linear range (µg mL^{-1})	0.5–18	0.05-5.0
Intercept, *a*	110.9	79.46
Slope, *b*	19.45	29.6
Correlation coefficient (R^2)	0.9995	0.9990
LOD (µg mL^{-1})	0.531	0.241
LOQ (µg mL^{-1})	1.77	0.806

Table 3. Accuracy and precision of the proposed method

Drug	Amount added (µg mL^{-1})	Recovery* %	RSD*
Tetracycline hydrochloride	5	103.99	1.19
	10	101.05	2.58
	15	97.80	0.79
Terbutaline sulphate	1	102.80	0.93
	2.5	98.38	3.87
	4	97.69	1.98

Mechanism of the reaction

The stoichiometry of the reaction was found as 1:2 ratios (drug/Eosin Y), confirming that one molecule of drug reacts with two molecule of Eosin Y. As seen in the chemical structures of drugs, tetracycline hydrochloride have two basic centres of primary and tertiary aliphatic amino groups which are involved in nucleophilic reactions. Thus the carboxylate group of Eosin Y can be attacked by these nucleophilic groups. Terbutaline sulphate structure composed of two molecules of terbutaline combined with sulphuric acid and each molecule have one basic centre of secondary primary amino group which are attacked by the carboxylate group of Eosin Y. Based on all these facts, the proposed mechanisms of these reactions pathway are shown in Figure 13.

Table 4. Assay of tetracycline hydrochloride and terbutaline sulphate drugs in tablet pharmaceutical formulations by the proposed and British Pharmacopoeia methods. ([a] Every reading is an average of five determinations for the proposed method and average of three determinations for British pharmacopoeia method. [b] provided from SDI Co. Iraq. [c] Manufactured by Mediotic labs Homs-Syria. [d] The results obtained by Ref. 31).

Procedure applied	Pharmaceutical preparation	Drug amount taken (μg mL^{-1})	Recovery [a] (%)	Drug constant found, mg	Average recovery content	Certified value, mg
	Tetracycline [b]	5	102.49	256.22	248.06	
	Capsule	10	98.79	246.97	t-test =1.61	250
Proposed		15	96.40	241.00	F-test= 0.102	
method	Asmanol [c]	1	105.68	5.28	5.07	
	tablets	2.5	98.83	4.94	t-test =1.12	5
		4	99.97	4.99	F-test= 1.34	
British	Tetracycline	250 mg	97.92	244.80	-	
Pharmacopoei	Capsule					250
a	Asmanol [d]	15 mg	99.21	14.88 mg	-	
	tablets					15

[a] Every reading is an average of five determinations for the proposed method and average of three determinations for British pharmacopoeia method. [b] provided from SDI Co. Iraq. [c] Manufactured by Mediotic labs Homs-Syria. [d] The results obtained by Ref. 31

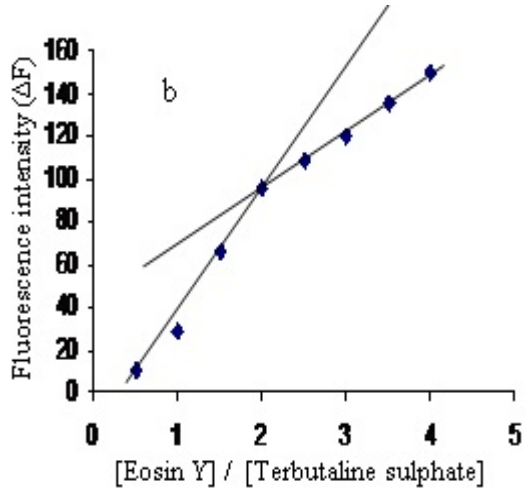

Figure 12a. Molar ratio plot for terbutaline sulphate (0.5 ml of 9.11×10^{-5}M) complexes with Eosin Y.

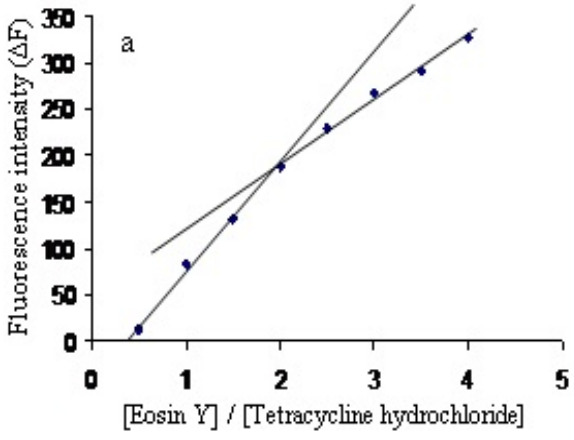

Figure 12b. Molar ratio plot for tetracycline hydrochloride (0.5 ml of 1.04×10^{-4}M)

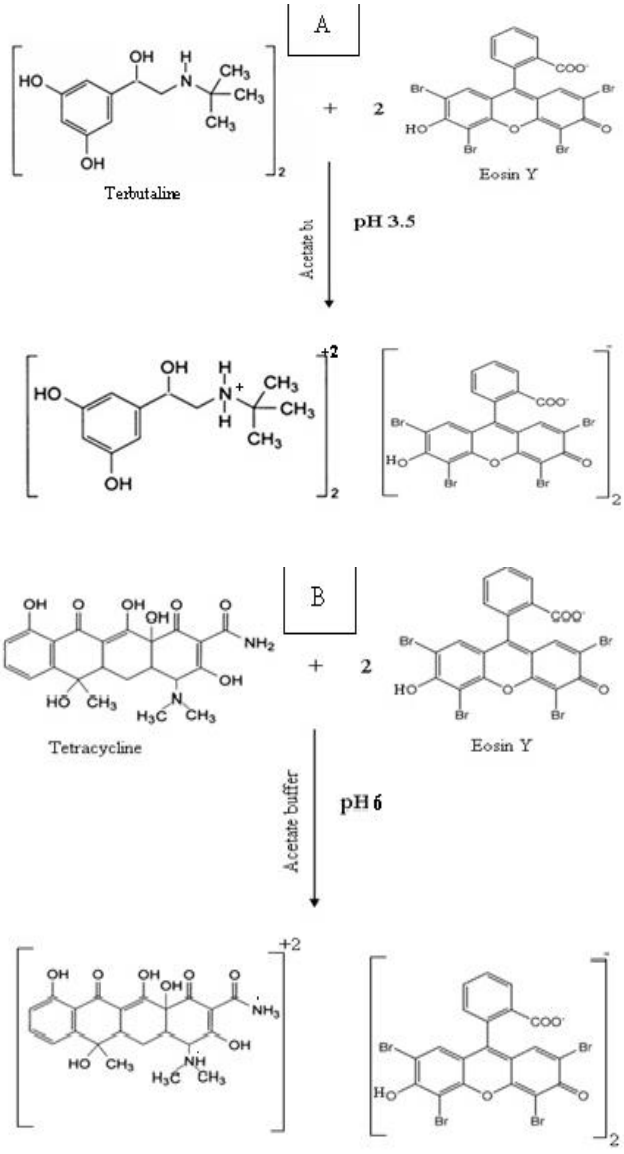

Figure 13. Proposed mechanisms for the reaction between terbutaline sulphate and tetracycline hydrochloride with Eosin Y.

Conclusion

In this study, direct, simple, and sensitive spectrofluorimetric procedure was developed and validated for determination of two drugs; tetracycline hydrochloride and terbutaline sulphate without interference from common excipients. The most important advantage of the method is rapid and inexpensive that the ion-pair formed is measured directly without need for pretreatment of the drug and extraction with organic solvent beside the use of water as diluting solvent. Hence, it can be applied for the routine quality control of the studied drug in its dosage forms.

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QUANTUM-CHEMICAL CALCULATION METHODS TO DETERMINE THE IONIZATION CONSTANTS OF N-SUBSTITUTED AMINO ACID DERIVATIVES

Faiz M. AL-Abady[a] and Noor H. Saeed[b]*

Keywords: amino acids derivates, computational chemistry, ionization constant.

A theoretical study was conducted to determine the ionization constants of N-substituted amino acid derivatives using HF, DFT, and MP2 calculation methods. The extent of compatibility of these methods was determined by discussing the theoretical variables calculated in the three methods mentioned above, the relationship between the calculated physical variables have been found to be theoretical and to determine their nature. These variables were then correlated with the known chemical values of amino acids as pK_a ionization parameters. The results obtained by this relationship were found to be good. This is indicated by the results of the statistical analysis across the correlation coefficient values. The theory that gave the best agreement between the values of the theoretical and the experimental ionization parameters were the MP2 method with good correlation coefficient (0.997) and standard error (0.162). As well as the large overlap between pK_a values calculated theoretically with practical values where the difference (0.008) gives the opportunity to apply these variables in other studies.

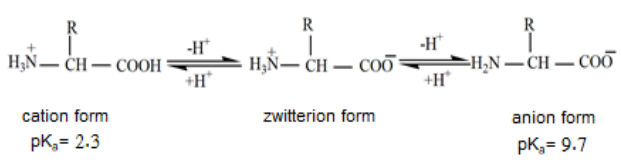
* Corresponding Authors
Tel.: +964 7515107216
E-Mail: nhsaeed@yahoo.com
[a] Chemistry Department, College of Science , University of Tikrit, IRAQ
[b] Chemistry Department, College of Education, University of Mosul, IRAQ

Introduction

Amino acids have a particularly important location in biology as basic building blocks of all proteins and contribute to cellular functions.[1]

The terms dissociation/protonation constants are referred to measure of acidic or alkaline force. The term ionization constant is called in the case of zwitterions.[2] Typically three types of amino acid ionization constants are defined: pK_a refers to the ionization of the carboxyl group, pK_b represents the ionic group ionization constant, and pK_c refers to the side group R, which may contains ionizable groups, and exist in the form of zwitter ions.[3]

Amino acids in a strong basic medium can behave as acids as they lose theirs proton and becomes anion. In the strong acidic medium, they can behave as bases and acquire proton and become positive ion (cation).[4] In neutral solutions, the concentration of zwitterions is very high and this behaviour can be illustrated by Scheme 1.



Scheme 1. Forms of amino acids in media of different acidities.

A list of amino acid derivatives investigated in this study is given in Table 1.

Table 1. Names of amino acids derivatives and their formula.

No.	Compound	Molecular formula
1	N-ethyl glycine	$CH_3CH_2NHCH_2COOH$
2	N-propyl glycine	$CH_3(CH_2)_2NHCH_2COOH$
3	N-n butyl glycine	$CH_3(CH_2)_3NHCH_2COOH$
4	N-isobutyl glycine	$(CH_3)_2CHCH_2NHCH_2COOH$
5	N-phenyl glycine	$PhNHCH_2COOH$
6	N-benzoyl glycine	$PhCONHCH_2COOH$
7	N-methyl alanine	$CH_3NHCH(CH_3)COOH$
8	N-ethyl alanine	$CH_3CH_2NHCH(CH_3)COOH$
9	N-n-propyl alanine	$CH_3(CH_2)_2NHCH(CH_3)COOH$
10	N,N-dimethylglycine	$(CH_3)_2NCH_2COOH$
11	N,N-diethylglycine	$(CH_3CH_2)_2NCH_2COOH$

Computational chemistry

Computational chemistry is one of the branches of physical chemistry along with quantum mechanics and molecular mechanics, whose purpose is to find the most important properties of the chemical compounds and compare them with the experimental values. It is one of the main branches of research used in analysis and diagnostics and support of experimental research.[7] Theoretical chemistry can be presented as a mathematical description of computational chemistry.[8,9] The most important of these variables is the electronic density, which represents the square wave function (), which indicates the possibility of an electron in the vicinity of the nucleus or on any atom in the molecule.[10] Other important variables include van der Waals forces, dipole-dipole, space disruptive energy and energy variables like HOMO and LUMO values. HOMO represents the least energy to remove electrons from the orbital outside the compound to the oxidizing state called ionization potential.

LUMO is the least possible energy needed to acquire an electron into the outer orbit and the compound changes to a reduced state called electronic affinity.[11] Some other variables such as hardness (η),[12] electronic chemical potential (μ)[13] and the global electrophilicity index (W)[14] are calculated from these energy variables with the help of following relationships.

$$\eta = 1/2(E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}}) \quad (1)$$

$$W = \frac{\mu^2}{2\eta} \quad (2)$$

$$\mu = 1/2(E_{\mathrm{LUMO}} + E_{\mathrm{HOMO}}) \quad (3)$$

Theoretical Calculations

The theoretical calculations have been completed in different ways such as (HF-6.31G (d)), (DFT- (B3LYP / 6.31G (d)) and (MP2-6.31G (d)) and using the (Chem office2015 Gaussian3 program) according to the following steps:

1. The molecular formula was drawn using the program (Chem. Draw)
2. Perform the process of preparing the molecule for calculations using the Clean Up directive
3. The energy reduction process was performed using the MM2 program.
4. The calculations were conducted according to each of the methods mentioned above.
5. Some variables related to the constants to be studied (e.g., charge, van der Waals, HOMO, LUMO) are taken from the program results

The variables that taken from step 4 were processed by SPSS package to obtain the best of these variables about the values to be calculated and the weight of each.

Results and Discussion

Theoretical Calculations

In this study, some of the physical variables of the amino acid derivatives were observed for the effective sites as shown in Figure 1. The finding of the relationship between them and the values of ionization constants by the methods mentioned above.

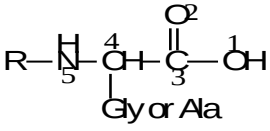


Figure 1. Proposed active sites for amino acid derivatives.

The purpose of selecting these methods is to compare them and to indicate the performance of each method through the accuracy of the calculations and their compatibility with the experimental values of pK_a. The choice of these methods is based on the most common and most widely used in this field of studies and their propensity to yield accurate results observed in the literature.[15-19]

Theoretical calculations of some physical variables of amino acid derivatives

Charge effect

Calculations of the Mulliken charge (which represent the difference between the negative electron density in orbits and the amount of positive protons in the nucleus) were calculated for five atoms at the centre of the interaction of amino acid derivatives using the basic calculation methods (MP2, DFT and HF). The results are given in Tables 2-4.

From the observation of the values of the charges in Tables 2-4 we see that they change in a certain pattern. This change can be explained if we take one of the compounds as an example of this change and explain it in detail and quantitative quantities. Taking into consideration the N-ethyl glycine we find the electron charge is concentrated on the C4-carbon atom because it is close to the amino group associated with the R group, while the electron charge decreases on the C3-atom near the O1 and O2 in the carboxyl group and the increase in the charge on O1, O2 and N5 is clearly observed. As expected, due to the electronegativity of these atoms with the electron abundance on these atoms, especially the presence of free electronic pairs, and reference to other amino acids, the value of the electronic charge on the C4-atom varies with the change in the nature of the group R linked to nitrogen Through its ability to draw and donate, during the observation of the movement of electrons on the atoms in the centre of the interaction can be concluded that the electronic movement, although the aliphatic systems it does not depend on the neighbouring atoms, but beyond it to reach further and this can be demonstrated by the decrease in the electron charge on C3-atom and its increase in the outer O1 and O2 atoms. It is different from what is expected if these groups were isolated. This change includes all methods (MP2, DFT and HF) With differences in charge values following the same pattern for each method.

The theoretical basis for this comparison is the extent of differences in the distribution of charges on atoms at the centre of the reaction (Figure 1). The greatest variation in the distribution of charges on atoms at the centre of the interaction is the sensitivity of the method adopted in this type of application. In order to better explain these differences, charges on O1, O2, C3, C4 and N5 in N-n-butyl glycine, determined by the three methods were plotted (Figure 2). The plots showed that the difference in the values of these charges, calculated by MP2 (1), is the most extensive when compared with the methods of HF (3) and DFT (2). This distinguishes HF and DFT from other methods and qualifies them for wider use and makes the results obtained are more accurate and consistent. This is in line with previous studies[21] in this field on the thiophene and furan derivatives because they gave significant differences between C-3 and oxygen which have high electronegativity.

Table 2. The values of physical variables of the amino acid derivatives calculated by MP2 method.

Compound No.	Charge on atom						S.E	Dipole-Dipol	VDW
	O1	O2	C3	C4	N5	H6			
1	-0.7107	-0.5691	0.7720	-0.1985	-0.7010	0.46971	0.1449	0.8626	1.7399
2	-0.7109	-0.5689	0.7713	-0.1973	-0.7103	0.4695	0.2201	0.8928	2.4189
3	-0.7108	-0.5696	0.7733	-0.1983	-0.7120	0.4693	0.2782	0.8578	3.1401
4	-0.7105	-0.5702	0.7741	-0.1968	-0.7150	0.4695	0.3502	0.8518	3.0536
5	-0.7070	-0.5780	0.7685	-0.1961	-0.8011	0.4737	0.2900	3.3964	5.8515
6	-0.7151	-0.5726	0.7823	-0.2177	-0.7879	0.4712	0.4253	-0.603	6.8529
7	-0.7076	-0.5628	0.7761	-0.0433	-0.6840	0.4669	0.2136	1.4979	1.8326
8	-0.7079	-0.5634	0.7746	-0.0345	-0.6975	0.4665	0.2764	1.5301	2.7540
9	-0.7228	-0.5667	0.7826	-0.0545	-0.6914	0.467	0.3338	1.5047	3.4710
10	-0.7160	-0.5633	0.7664	-0.2108	-0.5287	0.4683	0.4398	2.1946	3.3251
11	-0.7174	-0.5644	0.7713	-0.2164	-0.5534	0.4677	0.8671	2.2670	5.0292

O_1, O_2, C3 C4 = Charge on the carbonyl group in Coulomb, N5 Charge on the amine group in Coulomb, Steric S.E = Energy in Kcal mol^{-1}, VDW = Van Der Waals interaction, For names of the compounds see Table 1.

Table 3. The values of physical variables of the amino acid derivatives calculated by DFT method.

Compound No.	Charge on atom						S.E	Dipole-Dipole	VDW
	O1	O2	C3	C4	N5	H6			
1	-0.5738	-0.4576	0.5616	-0.1837	-0.53309	0.4159	0.1449	0.8626	1.7399
2	-0.5779	-0.4550	0.5671	-0.1889	-0.5451	0.4119	0.2201	0.8928	2.4189
3	-0.5781	-0.4539	0.5665	-0.1915	-0.5414	0.4141	0.2782	0.8578	3.1401
4	-0.5779	-0.4542	0.5655	-0.1884	-0.5422	0.4144	0.3502	0.8518	3.0536
5	-0.5620	-0.4576	0.5658	-0.1771	-0.6763	0.4175	0.2900	3.3964	5.8515
6	-0.5681	-0.4601	0.5965	-0.2261	-0.5866	0.4140	0.4253	-0.603	6.8529
7	-0.5709	-0.4605	0.5763	-0.0007	-0.5307	0.4092	0.2136	1.4979	1.8326
8	-0.5712	-0.4611	0.5739	0.0077	-0.5429	0.4089	0.2764	1.5301	2.7540
9	-0.5765	-0.4591	0.5892	-0.0203	-0.5458	0.4085	0.3338	1.5047	3.4710
10	-0.5697	-0.4524	0.5713	-0.1970	-0.3522	0.4103	0.4398	2.1946	3.3251
11	-0.5708	-0.4541	0.5753	-0.2124	-0.3620	0.4096	0.8671	2.2670	5.0292

Table 4. The values of physical variables of the amino acid derivatives calculated by HF method.

Compound No.	Charge on atom						S.E	Dipole-Dipole	VDW
	O1	O2	C3	C4	N5	H6			
1	-0.7228	-0.5686	0.7639	-0.1918	-0.6840	0.4754	0.1449	0.8626	1.7399
2	-0.7275	-0.5651	0.7684	-0.1974	-0.6972	0.4712	0.2201	0.8928	2.4189
3	-0.7274	-0.5647	0.7678	-0.1996	-0.6936	0.4734	0.2782	0.8578	3.1401
4	-0.7274	-0.5647	0.7678	-0.1996	-0.6936	0.4734	0.3502	0.8518	3.0536
5	-0.7151	-0.5726	0.7823	-0.2177	-0.7879	0.4712	0.4253	-0.603	6.8529
6	-0.7207	-0.5691	0.7779	-0.0462	-0.6719	0.4685	0.2136	1.4979	1.8326
7	-0.7207	-0.5691	0.7779	-0.0462	-0.6719	0.4685	0.2136	1.4979	1.8326
8	-0.7209	-0.5700	0.7756	-0.0349	-0.6884	0.4682	0.2764	1.5301	2.7540
9	-0.7108	-0.5482	0.7628	-0.0474	-0.6914	0.4651	0.3338	1.5047	3.4710
10	-0.7160	-0.5633	0.7664	-0.2108	-0.5287	0.4683	0.4398	2.1946	3.3251
11	-0.7057	-0.5472	0.7523	-0.2136	-0.5624	0.4661	0.8671	2.2670	5.0292

Table 5. Relation between spatial impedance energy and size of the substituent.

Compounds	**1**	**2**	**3**	**4**	**10**	**11**
R	Et	n-Pr	n-Bu	I-Bu	Me_2	Et_2
S.E	0.145	0.220	0.278	0.350	0.440	0.867

Spatial effect

The purpose of the vacuum arrangement is the geometrical form that amino acids can take and the distribution of atoms in the vacuum controlled by different factors: the first is the disability factor (increasing the particle's energy and decreasing its stability), which works to increases the electrons pushing the atoms of the disabled in the distant direction to alleviate the dissonance caused by these totals.

Table 6. Relation between vacuum impedance and size of the substituent.

Compounds	7	8	9
R	Me	Et	N-Pr
S.E	0.2136	0.2764	0.3338

The second effect is the spatial interference of different types, such as interference of van der Waals and hydrogen bonds. Based on the above, some theoretical variables were selected such as the energy of spatial disability (S.E.) and the power of van der Waals interactions (VDW 1-4). It was calculated as a model to describe the effect of the substituted groups in the reaction centre of the amino acids under study. The values of these variables are listed in Tables 2-4.

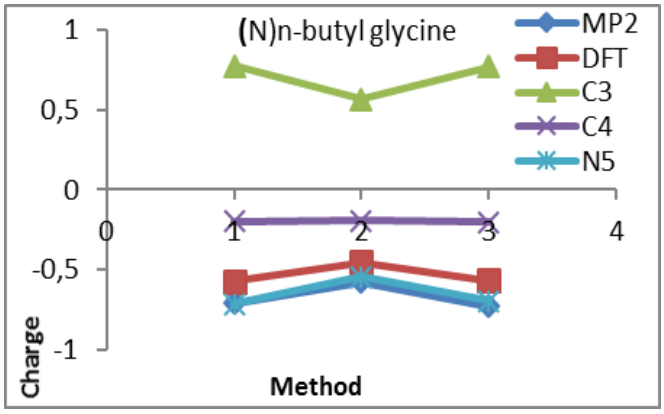


Figure 2. Charge on the reaction centre atoms of the N-butyl glycine compound by the three methods.

Table 7. Energy variables calculated theoretically for amino acid derivatives by MP2.

Compound No.	**HOMO**	**LUMO**	**η**	**μ**	***W***
1	-0.37381	0.18093	0.016766	-0.09644	0.27737
2	-0.37302	0.18137	0.016563	-0.09583	0.27720
3	-0.37263	0.18141	0.016499	-0.09561	0.27702
4	-0.37216	0.18063	0.016590	-0.09577	0.27640
5	-0.28984	0.14216	0.012621	-0.07384	0.21600
6	-0.34576	0.09444	0.035871	-0.12566	0.22010
7	-0.37722	0.17036	0.019536	-0.10343	0.27379
8	-0.37598	0.17301	0.018760	-0.10149	0.27450
9	-0.37866	0.17545	0.018631	-0.10161	0.27706
10	-0.36743	0.17874	0.016297	-0.09435	0.27309
11	-0.36265	0.17999	0.015371	-0.09133	0.27132

Table 8. Energy variables calculated theoretically for amino acid derivatives by DFT.

Compound No.	**HOMO**	**LUMO**	**η**	**μ**	***W***
1	-0.22267	0.01021	0.11644	-0.10623	0.048458
2	-0.22282	0.00865	0.11574	-0.10709	0.049541
3	-0.22087	0.00888	0.11488	-0.10600	0.048901
4	-0.22245	0.00800	0.11523	-0.10723	0.04989
5	-0.19883	0.00909	0.10396	-0.09487	0.043287
6	-0.25201	0.04065	0.14633	-0.10568	0.038161
7	-0.22700	0.00195	0.11448	-0.11253	0.055304
8	-0.22559	0.00315	0.11437	-0.11122	0.054078
9	-0.22721	0.00717	0.11719	-0.11002	0.051644
10	-0.22136	0.00670	0.11403	-0.10733	0.050512
11	-0.21764	0.00844	0.11304	-0.10460	0.048395

Table 9. Energy variables calculated theoretically for amino acid derivatives by HF.

Compound No.	HOMO	LUMO	η	μ	*W*
1	-0.37506	0.18243	0.27875	-0.09632	0.01664
2	-0.37589	0.18062	0.27826	-0.09764	0.017129
3	-0.37316	0.18104	0.2771	-0.09606	0.01665
4	-0.37393	0.18032	0.27713	-0.09681	0.016908
5	-0.28984	0.14216	0.216	-0.07384	0.012621
6	-0.34580	0.09443	0.22012	-0.12569	0.035883
7	-0.38089	0.17213	0.27651	-0.10438	0.019701
8	-0.37818	0.17467	0.27643	-0.10176	0.018729
9	-0.37860	0.17586	0.27723	-0.10137	0.018533
10	-0.36743	0.17874	0.27309	-0.09435	0.016297
11	-0.35338	0.19562	0.2745	-0.07888	0.011333

Table 10. Correlation coefficient values for the relationship between the calculated variables of MP2.

Variables	O1	O2	C3	C4	N5	H6	SE	*VDW*	*HOM*	*LUM*	η	μ	*W*
O1	1												
O2	0.240	1											
C3	0.390	0.009	1										
C4	0.055	0.494	0.445	1									
N5	0.428	0.763	0.410	0.059	1								
H6	0.304	0.945	0.233	0.649	0.603	1							
SE	0.522	0.215	0.081	0.325	0.568	0.142	1						
VDW	0.256	0.549	0.175	0.411	0.260	0.570	0.535	1					
HOM	0.264	0.773	0.264	0.353	0.454	0.836	0.081	0.712	1				
LUM	0.003	0.533	0.432	0.183	0.562	0.553	0.058	0.790	0.571	1			
η	0.145	0735	0.101	0.301	0.574	0.782	0.078	0.848	0.883	0.890	1		
μ	0.285	0.238	0.755	0.175	0.134	0.283	0.023	0.109	0.437	0.489	0.037	1	
W	0.214	0.089	0.704	0.011	0.335	0.073	0.037	0.451	0.043	0.794	0.431	0.916	1

Table 11. Multiple regression analysis of the variables used to calculate pK_a values of amino acid derivatives for all methods.

Method &Group	Parameter	*SE*	*R*
MP2	C4,N5, μ	0.162	0.997
DFT	C4,N5,HOM	0.397	0.994
HF	C4,LUM,W	0.471	0.985

An examination of Tables 2-4, revealed that the value of the spatial impedance energy increases with the size of the substituted group (Table 5).

In the substituted alanine, the value of vacuum impedance also increases by increasing the size of the compensated group in the following order (Table 6).

In the N-benzoylglycine compound, the value of vacuum impedance is greater than that of N-phenylglycine due to the presence of the carbonyl group, which makes it more stable.

As for the values of the impact of dipole moment, we noticed that it is also affected by the nature of the substituent found on the amino acids, and increase the size of the substituted and its polarities in a manner consistent with the effects of spatial disability. It is observed that the values of van der Waals interaction are minimum in the N-ethylglycine. This energy increases with an increase in the size of the substituent, which is the result of a type of van der Waals power as a result of interference resulting from spatial disability, and this is consistent with the values of spatial disability.

Similar results are obtained by other methods, MP2 and HF also. Although the values vary, the pattern of change is identical by the remaining methods. This confirms that the above results are in line with known chemical bases.

The values of the energy variables calculated theoretically for amino acid derivatives by three different methods are given in the Tables 7-9. The relationship between each of the variables was found in the MP2 method as a model and for amino acid derivatives. The results of these ratios were included in the Table 10.

The tables showed that there are good relations between some variables vary by proximity and distance from each other in terms of vacuum location and electronic payment, these relations vary in values according to the different method.

Table 12. The theoretical and calculated pKa values and the difference between the amino acids derivatives by the three methods.

Compound No.	MP2			DFT			HF		
	pK_a^*	pK_a^{**}	ΔpK_a	pK_a^*	pK_a^{**}	ΔpK_a	pK_a^*	pK_a^{**}	ΔpK_a
1	2.34	2.3892	-0.0492	2.34	2.3867	-0.0467	2.34	2.298062	0.04193
2	2.35	2.3813	-0.0313	2.35	2.4203	-0.0703	2.35	2.341551	0.00844
3	2.35	2.3798	-0.0298	2.35	2.3513	-0.0013	2.35	2.313705	0.03629
4	2.35	2.3882	-0.0382	2.35	2.4020	-0.0520	2.35	2.327509	0.02249
5	1.83	1.7791	0.0509	1.83	1.7759	0.0541	1.83	1.82977	0.00023
6	3.62	3.5785	0.0415	3.62	3.5652	0.0548	3.62	3.597436	0.02256
7	2.22	2.2426	-0.0226	2.22	2.2077	0.0123	2.22	2.275847	-0.05585
8	2.22	2.1800	0.04	2.22	2.1632	0.0568	2.22	2.185033	0.03496
9	2.21	2.2193	-0.0093	2.21	2.2740	-0.0640	2.21	2.193352	0.01664
10	2.08	2.0613	0.0187	2.08	2.0767	0.0033	2.08	2.290829	-0.21083
11	2.04	2.0096	0.0304	2.04	1.9906	0.0494	2.04	1.948099	0.09190

pK_a^* = Experimental Values, pK_a^{**} = Calculated Values, ΔpK_a = $pK_a^* - pK_a^{**}$

For example, in Table 10 correlation coefficient values were in the MP2 theory between the C3 atom and the chemical voltage is 0.755. Its value is also relatively good with the energy variables represented by VDW and hardness interference on the one hand and chemical and electrolyte on the other i.e. 0.848 and 0.916, respectively.

The following equation represents the model used to calculate pKa values in the MP2

$$pK_a=-2.529+(-2.300*C4)+(-1.670*N5)+(-34.137*\mu)$$

In considering the above table, it can be noted that the correlation coefficient obtained in MP2 theory was high and close to one. This indicates the importance of using these variables in calculating the value of pK_a and comparing them with the values obtained from the literature The standard error (S.E) of the relationship between the values of ionization constants and the theoretically calculated variables in simple analysis was small for the amino acid derivatives in MP2 theory where they were better than the rest of the theories.

Theoretical calculation of pK_a ionization parameters for amino acid derivatives

The results of the statistical analysis were used to determine the important variables used to calculate the ionization parameters in the calculation of the theoretical values and the differences from the experimental values taken from the literature[27-23] and the three methods as shown in Table 12.

It is observed that there is a large congruence between the values of the pKa ionization constants calculated theoretically with the experimental values obtained from the literature for the MP2 method of amino acid derivatives. This is an indication of the accuracy of the variables used to calculate these values. MP2 theory gave the most accurate results in the convergence of values between the constants of theoretical ionization and the constants of ionization obtained from literature and the sequence of theories in terms of preference is DFT<MP2<HF.

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RELATIONSHIP OF SEROTONIN TO NEURON SPECIFIC ENOLASE IN SERUM SAMPLES OF PATIENTS WITH ADVANCED STAGES OF CANCER

Noor Sabah Matlab[a] and Rasha Hasan Jasim[a]

Keywords: Cancer; Neuron-Specific Enolase (NSE); Serotonin; Chemotherapy; Radiotherapy

Cancer is heterogeneous disorders characterized by cellular different genetic alterations and diverse clinical behaviours results from uncontrolled division leads to loss of control of the cells growth. Neuron Specific Enolase(NSE) is considered as a marker of many diseases such as brain damage (traumatic brain injury), stroke and anoxic encephalopathy after cardiac arrest. Generally, in adults NSE accepted as a marker protein in the brain. Serotonin exhibits a growth stimulatory effect on several types of carcinoma, carcinoid and other tumor cells. In contrast, a few data are available on serotonin involvement in cancer cell migration and metastatic processes. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma. 201 patients with malignant tumors, 74 patients with different benign tumors and, 83 healthy individuals were enrolled in the present study. Our results show a significant increase (p = 0.011, and 0.043) of serum serotonin levels in malignant tumors group when compared with those of benign tumors (as a pathological control) group, and healthy individuals groups; respectively. No such results were shown when the two control (benign tumors and healthy individuals) groups were compared together. While evaluation of the NSE concentrations revealed a significant decrease in patients with malignant tumors when compared with those of benign tumors (p = 0.028), and healthy individuals (p< 0.000). According to ANOVA test, Same variations (p < 0.000) were obtained when benign tumors and healthy controls groups were compared together. Results shows a significant decrease in the serotonin concentration (p < 0.05) as well as the NSE (p < 0.01) levels, correlation between the levels of the two examined parameters has been negative and statically acceptable (r = - 0.723 at p<0.05) at malignant tumor patients group after treatment by chemotherapy or radiotherapy. Before treatment with chemotherapy or radiotherapy, negatively significant correlation (r = - 0.792 at p < 0.001) was observed for the concentrations of Serotonin to the NSE in the sera of malignant tumor patients group, while no such correlations were noted at this relation examined in the benign tumor patients. Serotonin and NSE correlations together can be used as primary diagnostic tools for distinguishing between cancerous and benign tumors, this correlation increases the sensitivity of the two biomarkers together comparing to measure everyone alone.

* Corresponding Authors
E-Mail: dr.rashahussainee@yahoo.com
[a] Department of Chemistry-Factually of Education for Girls-University of Kufa-Iraq

Introduction

Cancer is a genetic disorder consequent uncontrolled and abnormal genetic changes,[1] The prime cancerous cells formed by growth resemble the parent, but as cancer progresses they lose the exterior and function of the parent cell, this dysfunction cells will become life menacing if they left unchecked.[2] Cancer is the main cause of death in economically developed countries and the second leading cause of death in developing countries. The heaviness of cancer is increasing in economically developing countries in consequence of growth and population aging also increasingly, an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and type of diets.[3]

Neuron Specific Enolase (NSE)is the glycolytic enzyme when it converts 2-phosphoglycerate into phosphoenol-pyruvate (2-phospho-D-glycerate hydrolase) "**E.C: 4.2.1.11**". It comprises three distant subunits α, β, and γ, these subunits might organize spatial as γ-γ and α-γ dimeric forms of enolase. The number of the amino acid is 433 residues and sizes of monomer and dimer differ in molecular weight 39,45, 47, and that of the dimer 7,78, 8, 95 and 96 kDa.[4-9] During the embryonic stage NSE is manufactured in the neural and lung tissue,[10] while, in healthy adults, it is synthesized in neuroendocrine cells.[11] Normally, it is found in blood and cerebrospinal fluid.[10,12] It was first considered that the gene coding for NSE was restricted to neurons and that it was only present in the central nervous system. In 1978 researches have shown that NSE is present in peripheral and central neuroendocrine cells, named APUD (amine precursor uptake and decarboxylation) cells. Moreover, studies have extended to involve immune-histochemical and extraction techniques.[13-15] When NSE passes into the extracellular compartment and the bloodstream causes structural damage in neuronal brain cells. In adult and pediatric study, NSE was considered as a marker of intracranial injury and found in the serum of traumatic brain damage.[16] NSE was shown to be increased in cerebrospinal fluid and blood as a result of injury due to reasons, such as cardiac arrest, open heart surgery, tonic-clonic seizures, epilepsy, and Creutzfeldt-Jakob disease.[17] In addition to that, in the liver diseases, erythrocytes, and benign lung tumor; the concentration of NSE increased (grow up to 20 μg L^{-1}).[10] NSE presents in a wide variety of APUD neoplasms or APUDomas including islet tumors of the pancreas, gastronomes, VIPomas, medullary carcinoma of the thyroid, pheochromocytoma, and small-cell carcinoma of the lung (SCLC).[15] According to that, NSE

was shown to be a valuable tumor marker for cancers of a neuroendocrine type such as small-cell lung cancer (SCLC), neuroblastoma, carcinoid tumors, melanoma, seminoma, Merkel cell carcinoma, medulloblastoma or retinoblastoma.[17] NSE is considered as a marker of many diseases such as brain damage (traumatic brain injury), stroke and anoxic encephalopathy after cardiac arrest. Generally, in adults NSE accepted as a marker protein in the brain.[18] It is found in neurons and neuroendocrine tissues as a neuronal form of the glycolytic enzyme enolase.[13]

In the late of 1940 serotonin (5-hydroxytryptamin "5-HT") as a neurotransmitter was discovered in the central nervous system(CNS) of animals,[19] after that; exactly at 1950 heterogeneous serotonin receptors in rat brain was discovered.[20,21] In mammals serotonin produced principally by enterochromaffin cells. It is found in the gut and stored within blood platelets. In the brain, serotonin is produced within axon terminals. Serotonin is released in response to an action potential and then diffuses across the synapse to activate postsynaptic receptors. They are specialized groups of cell bodies known as the raphe nuclei, located in the brainstem reticular formation.[22] Serotonin has an effect on the number of physiologic and behavioural function, It plays a number of very important roles in normal brain functions, which include modulation of mood states, memory, emotion, anxiety, endocrine effects appetite, hunger, aggression, cognition, gastrointestinal function, emesis, endocrine function, motor function, perception, neurotrophic, sensory function, sex, sleep and vascular function, and many others.[19,23] Serotonin exhibits a growth stimulatory effect on several types of carcinoma, carcinoid and other tumor cells. In contrast, a few data are available on serotonin involvement in cancer cell migration and metastatic processes. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma.[24] Serotonin used in oncology as a tumor marker of gastrointestinal carcinoid, hepatic and ovarian carcinoid.[24] In addition to, serotonin can be used as a specific tumor marker for gastrointestinal tumors of the pancreatic islet cells and intestinal tract.[23,25] Serotonin is synthesized by conversion L-tryptophan into 5-hydroxytryptamine in the body by using two catalyze factors: they are tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase.[19,26] The chemical structure of serotonin have comprised of a basic amino group separated from an aromatic nucleus by a two carbon aliphatic chain. In mammals, serotonin is biosynthetically derived by two enzymatic steps: (1) ring hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase, the rate-limiting step, and (2) side chain decarboxylation by aromatic amino acid decarboxylase.[24,22,23] These processes occur in a number of systems body such as immune system cells and gastrointestinal tract (GIT), central and peripheral nervous system.[27-29] GI contains 90 % of serotonin and it is synthesized basically in enterochromaffin cells and enteric neurons of submucous and myenteric plexus layer.[29] When the serotonin levels fall in the brain leads to a large number of the emergence of foul bad.[30] While serotonin function increases in humans to strengthen the qualities of the positive behaviour and the desired, whenever dropped the serotonin levels increased the emergence of aggressive behaviour.[31,32]

Materials and Methods

During the period from the beginning of March 2016 to the end of September 2016; 358 individuals were enrolled in the present study and classified into three groups. The first group involved 201 patients with different malignant tumors, while the second group included 74 patients underwent benign tumors were used as a pathological control, and the last group included 83 healthy individuals. The enrolled patients (malignant and benign tumors), were collected from several public and private hospitals in addition to centers in Al-Najaf Al-Ashraf governorate; involved: Al-Sadder Medical City, Al-Zahra Teaching Hospital, Al- Ameer Privet Hospital, Al-Ghadeer Hospital, Middle Euphrates Cancer Center, and Daily Specialized Najaf Clinic. Patients with malignant tumors were the basic group of the present study. The cancerous patient's group were classified into six general subgroups (Breast, Lung, Brain, Bladder, Lymphoma, and Acute Lymphocytic Leukemia (ALL)) according to the cases that have been followed during treatment with chemotherapy or radiotherapy.

Five milliliters of venous blood samples were collected from the patients and healthy individuals, after fasting period more than eight hours. Samples were allowed to clot at lab temperature, centrifuged at 5000 g for 5 minutes. Sera were collected and divided into two parts: the first was used for evaluating oxidative stress parameters, and the second part was used for tests of the immunoassay, then these parts were stored at -18˚C until used. Sandwich-Enzyme-Linked Immune Sorbent Assay (Sandwich-ELISA) method was applied to evaluate Serotonin and Neuron Specific Enolase (NSE) concentration in the serum.

Results and Discussion

Levels of serum Serotonin Concentration were measured in the three study groups; malignant and benign tumors' patients as well as healthy control individuals, at diagnosis and before treatment with chemotherapy or radiotherapy or the two types together. Table 1shows a significant increase (p=0.011, and 0.043) of serum serotonin levels in malignant tumors group when compared with those of benign tumors (as a pathological control) group, and healthy individuals groups; respectively. No such results were shown when the two control (benign tumors and healthy individuals) groups were compared together.

Table 1. Levels of serotonin concentration (ng mL^{-1}) in sera of tumoral patients and controls subjects (mean ± S.D.)

Groups (n)	Serotonin, ng mL^{-1}, mean±S.D.	Range	*P*
Malignant (201)	1.315±0.622	0.203-2.721	0.011, malignant vs benign
Benign (74)	0.856±0.594	0.152-2.496	0.043, malignant vs healthy
Healthy (83)	0.895±0.606	0.031-2.113	0.858, benign vs healthy

Measurement of the Neuron Specific Enolasein the various study groups revealed a significant decrease in patients with malignant tumors when compared with those of benign tumors (**p = 0.028**), and healthy individuals (*p* <0.000). Same variations (**p < 0.000**) were obtained when benign tumors and healthy controls groups were compared together (Table 2).

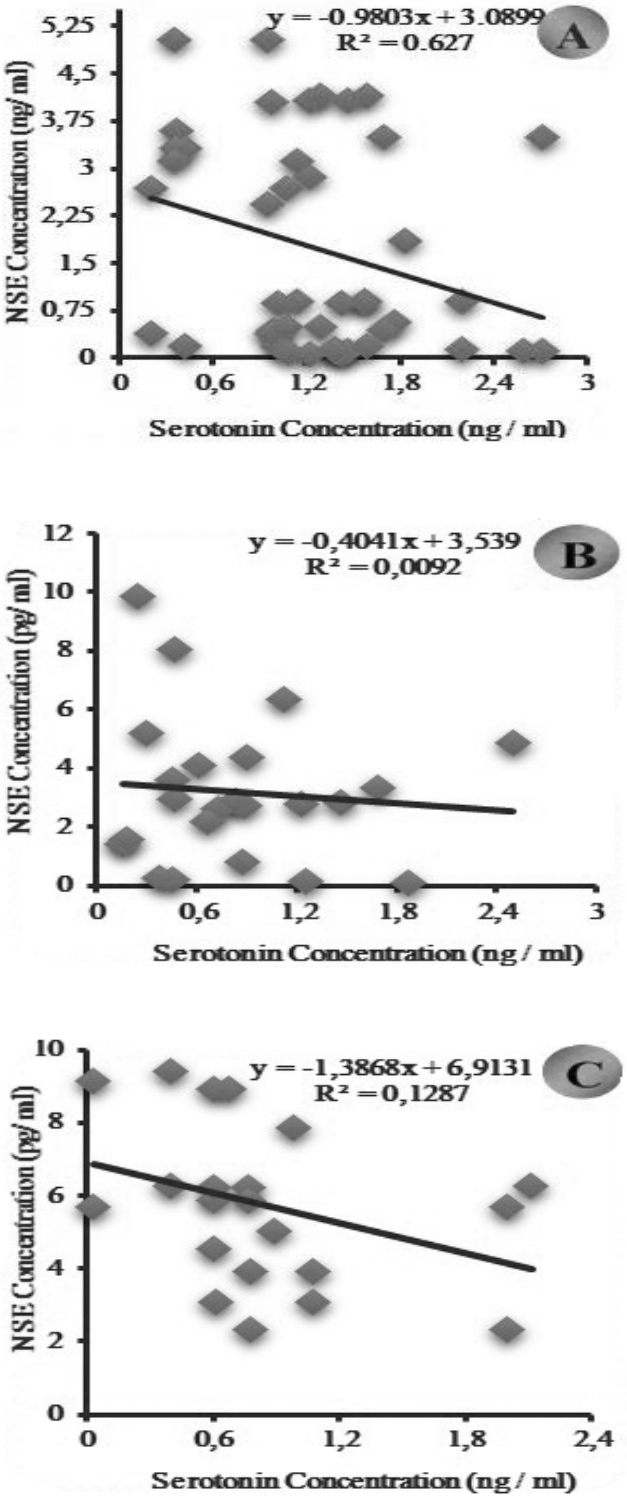

Figure 1. Relationship of serotonin concentration to Neuron Specific Enolase in the sera samples of (A) malignant tumor patients, (B) benign tumor patients, and (C) healthy control individuals

Table 2. Levels of neuron specific enolase concentration (ng mL^{-1}) in sera of tumoral patients and controls subjects (mean±S.D.)

Groups (*n*)	NSE conc. pg mL^{-1}, mean±S.D.	Range	p
Malignant (201)	1.801±1.623	0.060-5.020	0.028, malignant vs benign
Benign (74)	3.193±2.500	0.114-9.884	0.000, malignant vs healthy
Healthy (83)	6.237±2.445	2.310-9.408	0.000, benign vs healthy

Linear Regression Analysis "Pearson's Correlation" was used to analyze the results of Serotonin and Neuron Specific Enolase.

Before treatment with chemotherapy or radiotherapy, negatively significant correlation (r = - 0.792 at p < 0.001) was observed for the concentrations of Serotonin to the Neuron Specific Enolase in the sera of malignant tumor patients group as shown in Figure 1A, while no such correlations were noted at this relation examined in the benign tumor patients (Figure 1B, as well as; healthy controls as shown in Figure 1C.

Despite the recorded significant decrease in the serotonin concentration (p<0.05) as shown in Figure 3.2 as well as the Neuron Specific Enolase (p<0.01) as shown in Figure 3.4 levels, correlation between the levels of the two examined parameters has been negative and statically acceptable (r=-0.723 at p<0.05) at malignant tumor patients group after treatment by chemotherapy or radiotherapy, as illustrated in Figure 2.

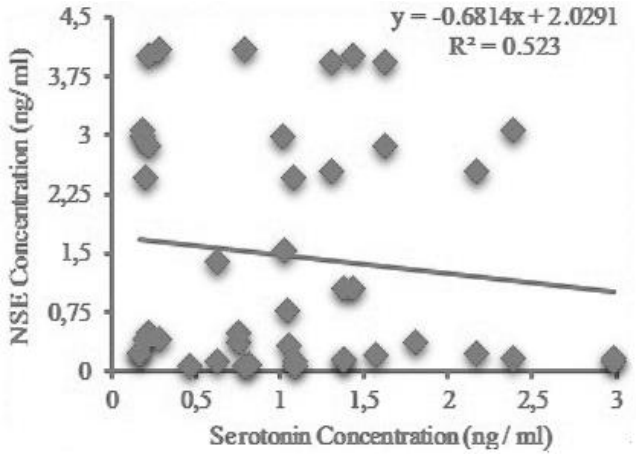

Figure 2. Relationship of Serotonin Concentration to Neuron Specific Enolase in The Sera Samples of Malignant Tumor Patients after at Least two Dosages of Treatment

According to the present results (**Figures 1** and **2**), correlation of Serotonin and Neuron Specific Enolase together increases the possibility of using them together as primary diagnostic tools for distinguishing between cancerous and benign tumors. This correlation increases the sensitivity of the two biomarkers together comparing to measure everyone alone. Moreover, due to the relationship

of serotonin and Neuron Specific Enolase is remained statistically significant in the samples of malignant tumors which are subjected to chemotherapy or radiotherapy, so testing of the two parameters together as follow-up tools became possible for noting the cellular changes occurring during and after treatment. Literatures survey didn't provide real help in tracing a relationship that links serotonin and neuron-specific enolase in cases of cancers.

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SPECTROPHOTOMETRIC DETERMINATION OF THE IONIZATION CONSTANTS OF METHIONINE, CYSTINE AND CYSTEINE

Hamad M. Adress Hasan[a]*, Ibrahim H. Habib[b] and Ahlam M. Ali[a]

Keywords: amino acids, ionization constant, spectrophotometry, cystine, cysteine, methionine.

A simple, rapid and sensitive method has been applied on three amino acids viz., methionine, cystine and cysteine, to estimate the ionization constant. Spectrophotometric measurements were carried out on the absorbance of each amino acid at different pH values. The results showed that the cystine and cysteine have one pK_a, while the methionine gave two different pK_a values by using this method.

* Corresponding Authors
E-Mail: Drhamadmhasan85@yahoo.com
[a] Chemistry Department, Faculty of Science, Omar El – Mukhtar University, Libya.
[b] Al-Dogy Research Center, Cairo, Egypt.

INTRODUCTION

Amino acids may have positive, negative, or zero net charge. Charged and uncharged forms of the ionisable COOH and NH_3^+ weak acid groups exist in solution in the following protonic equilibria.

$$\underset{(A)}{RCOOH} \leftrightarrows \underset{(B)}{RCOO^- + H^+} \quad (1)$$

$$RNH_3^+ \leftrightarrows RNH_2 + H^+ \quad (2)$$

While both RCOOH and RNH_3^+ are weak acids, RCOOH is a far stronger acid than RNH_3^+, at physiologic pH 7, carboxyl groups exist almost entirely as $RCOO^-$ and amino groups predominantly as RNH_3^+ form.

Molecules that contain an equal number of ionizable groups of opposite charge and that therefore bear no net charge are termed zwitter ions. Amino acids in blood and most tissues thus should be represented as (B). Structure A cannot exist in aqueous solution because at any pH low enough to protonate the carboxyl group the amino group would also be protonated.[1] Similarly, at any pH sufficiently high for an uncharged amino group to predominate, a carboxyl group will be present as $RCOO^-$. The uncharged representation (A) is, however, often used for reactions that do not involve protonic equilibrium.

The acid strengths of weak acids are expressed as their pK_a. The net charge on an amino acid-the algebraic sum of all the positively and negatively charged groups present-depend upon the pK_a values of its functional groups and on the pH of the surrounding medium. Altering the charge on amino acids and their derivatives by varying the pH facilitate the physical separation of amino acids, peptides and proteins.[2] The isoelectric species is the form of a molecule that has an equal number of positive and negative charges and thus is electrically neutral. The isoelectric pH, also called the pI is the pH midway between pK_a values on either side of the isoelectric species. For amino acids such as alanine that has only two dissociating groups, there is no ambiguity, the first pK_a (COOH) is 2.35, and the second pK_a (NH_3^+) is 9.69, the isoelectric pH (pI) of alanine is thus 6.02.

The environment of a dissociable group affects its pK_a, the pK_a values of the ionisable groups of free amino acids in aqueous solutions provide only an approximate guide to the pK_a values of the same amino acids when present in proteins. A polar environment favors the charged form, $RCOO^-$ or RNH_3^+, and nonpolar environment favors the uncharged form, RCOOH or RNH_2.[3]

A nonpolar environment thus raises the pK_a of the carboxyl group (making it a weaker acid) but lowers that of a protonated amino group (making it a stronger acid). The presence of adjacent charged groups can reinforce or counteract solvent effects. The pK_a of a functional group thus will depend upon its location within a given protein. Variations in pK_a can in compass whole pH units, pK_a values that diverge from those listed by as much as three pH units are common at the active sites of enzymes. An extreme example, a buried aspartic acid of thioredoxin has a pK_a, above 9 a shift over six pH units.[2]

Experimental

A stock solution of cysteine (10^{-4} mol) was prepared by dissolving 0.00302 g of cysteine in distilled water by gentle heating till complete dissolution. The solution was cooled and diluted to 250 mL. A stock solution of cystine (10^{-4} mol) was prepared by dissolving 0.0060 g of cystine in distilled water by gentle heating till complete dissolution. The solution was cooled and diluted to 250 mL. A stock solution of methionine (10^{-3} mol) was prepared by dissolving 0.0373 gm of methionine in 250 mL distilled water. More dilute solutions were prepared by diluting the stock solutions.

Buffer solutions

The buffer used was of the universal type (99) by taking 100 mL of an acid mixture containing 0.04 mol of H_3BO_4, H_3PO_4 and CH_3COOH acids and adding the required volume of 0.2 N NaOH to give the desired pH (Table 1).

Table 1. Constituting Universal buffer.

No.	pH	No.	pH
1	2.374	7	8.0
2	3.100	8	9.299
3	4.69	9	10.487
4	5.107	10	11.099
5	6.385	11	11.990
6	7.482		

Determination of pKa metal complexes by half height method

This method [3] depends on the fact that the limiting absorbance (A_1) represents the complete conversion of the compound from one form to other. Since pK_a is equal to the pH value at which the two forms exist in equivalent amounts, the pH corresponding to half the height of the absorbance-pH curve, $A_{½}$ is equal to pK_a. The $A_{½}$ value is given by the relation

$$A_{½} = (A_1 - A_{min}/2) + A_{min} \quad (1)$$

where

A_1 = maximum absorbance,

A_{min} = minimum absorption.

The absorbance of the amino acid was measured by a spectrophotometer type 800 DU.

Results and Discussion

The absorption spectra were recorded to investigate the spectral properties of the species likely to exist in such media and to determine the ionization constant (pKa) values of the acidic groups present.

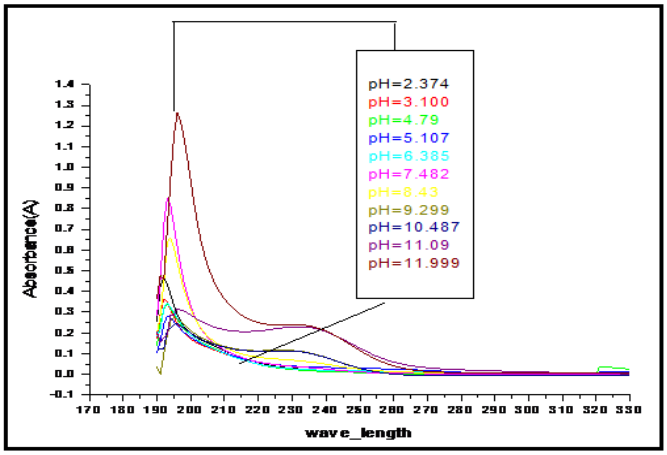

Figure 1. Electronic spectra of $1.3x10^{-4}$ mol MET at different pH.

Britton – Robinson universal buffers, were used to control the pH over the range (2.5- 12.0). From the Figures (1, 2 and 3), it is apparent that the maximum absorption of the ligand increases as pH of the buffer increases. The values of the maximum absorptions of the investigated ligands at different pH values are listed in table 2. These bands are due to absorption of the non- ionized form liable to exist in such solutions and may be assigned to $\pi - \pi^*$ electronic transition within the ligand molecule influenced by intermolecular charge transfer.[4] The

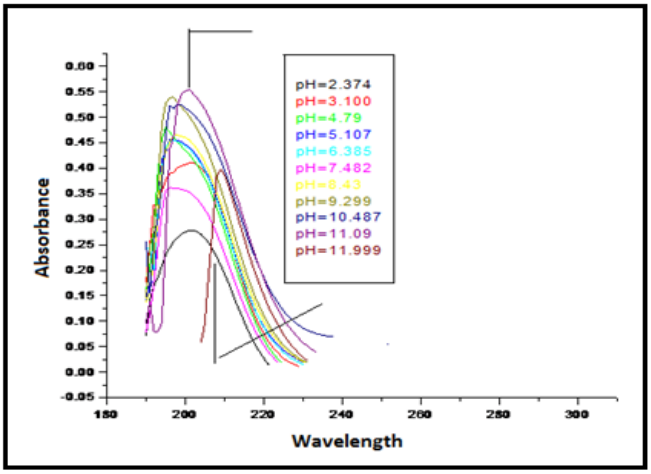

Figure 2. electronic spectra of $3x10^{-4}$ mol cystine at different pH.

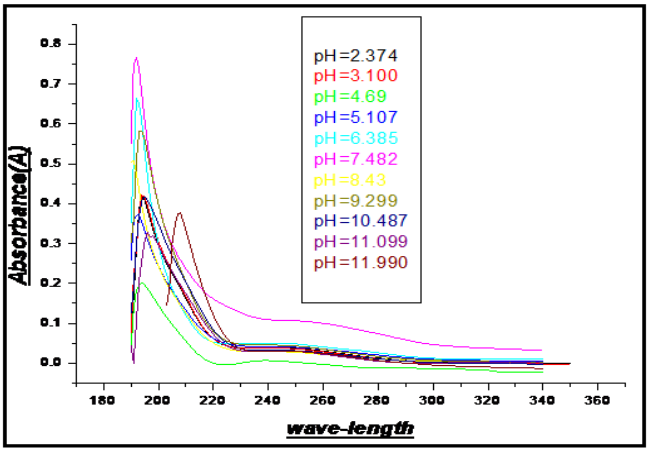

Figure 3. electronic spectra of $3x10^{-4}$ mol cysteine at different pH.

Table 2. Absorptions of the amino acids at different pH values.

pH	Absorbance		
	Cysteine	Cystine	Methionine
2.374	0.4654	0.4185	0.2782
3.100	0.3641	0.4218	0.4103
4.69	0.3427	0.2016	0.4802
5.107	0.2796	0.3719	0.4568
6.385	0.3463	0.6651	0.4549
7.482	0.8528	0.767	0.3609
8.43	0.6582	0.5088	0.4651
9.299	0.2759	0.5818	0.5388
10.487	0.2443	0.4183	0.5261
11.099	0.3182	0.3281	0.5541
11.990	1.2665	0.3777	0.3992

spectra in alkaline solutions are characterized by the presence of a strong band absorbing maximally at the same range, which may be assigned to the absorption of the ionized form liable to exist at high pH values as a result of acid base equilibrium. It has been noticed in this investigation that the absorption bands assigned to the ionized form increase gradually by increasing of pH, attaining the maximum value at pH nearly 12.0.

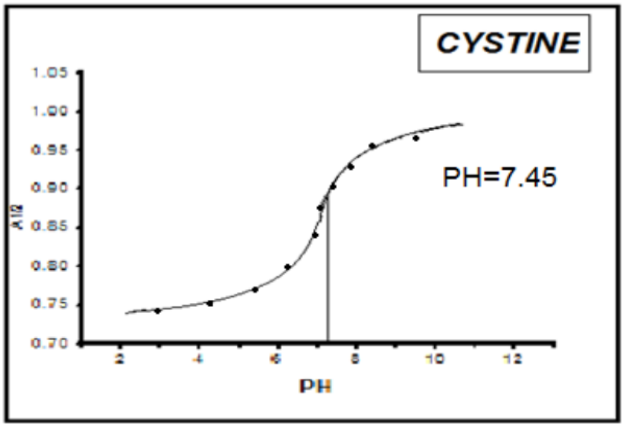

Figure 4. Absorbance and pH relationship for cystine.

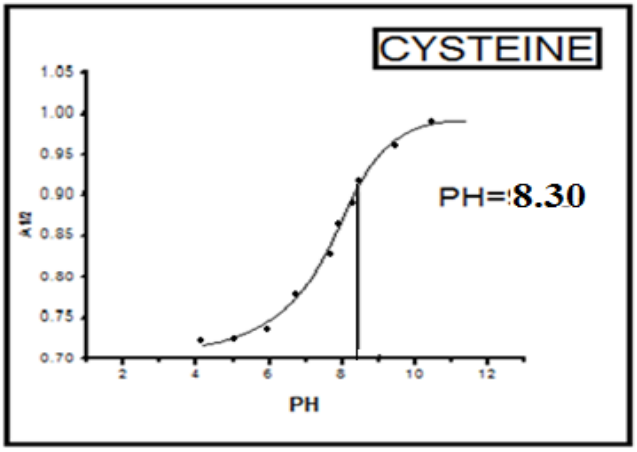

Figure 5. Absorbance and pH relationship for cysteine.

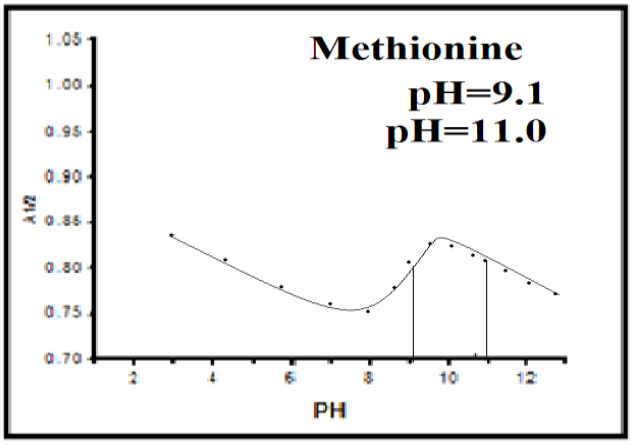

Figure 6. Absorbance and pH relationship for methionine.

The absorbance-pH curves show that the absorbance attains a limiting value at the extreme pH values in highly acidic or alkaline solutions indicating the existence of only one ionization step which is the ionization of NH_2 or COOH groups.

The variation of absorbance with pH is used for the calculation of ionization constants (pKa values) of the investigated ligands using the half height method.[4] The ionization of strong acidic carboxylic group is apparent at lower pH value, due to the high stability of the corresponding anion by resonance. They do not impart any spectral changes as the ionizable proton is not conjugated with the π–system of the molecule. In case methionine two maximal bands at 200 and 235 nm appeared. The two maxima increase with an increase in pH.

In the case of cystine one maximal band is apparent at 200 nm. This band becomes more intense with the increase of pH. On the other hand, in solution with pH > 9, the position of the maximum absorption band is red shifted with the increase of pH. This is an indication of the formation of an anionic species in alkaline solutions. The observations in case of cysteine are similar to those in cystine with two maxima at 200 and 235 nm and an increase in absorbance with an increase in pH.

The absorbance values of the studied amino acid are given in table (2) and shown in figures 4, 5, and 6.

Conclusions

1. Only one pK value was observed spectrophotometrically for cystine and cysteine.

2. In the case of cystine, the pK value obtained spectrophotometrically is 7.45 units. The sample must be dissolved in an acid for spectral measurements. The variation in the media from water to acid for solubility and the insolubility of the cystine zwitterion in aqueous solution leads to differences in the pK values.[5,6] The pKa of cysteine was 8.30.

3. In the case of methionine, two pK values are obtained spectrophotometrically, 8.96 and 11.030. The first pK is attributed to the deprotonation of the carboxylic group.[7] The second pK is smaller by 0.3-0.6 units than those obtained by pH-measurements and this may be due to protonation of zwitterions.[7]

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EVALUATION OF DISSOLUTION OF RIFAXIMIN AND ITS IMPORTANCE

Ana Carolina Kogawa[a]* and Hérida Regina Nunes Salgado[a]

Keywords: Antibiotic, dissolution, quality control, rifaximin, tablets.

Rifaximin, an oral antibiotic marketed as tablets, does not have dissolution method described either in official compendiums or literature. This lack of knowledge blinds the real rate of drug availability. Thus, all potentialities of the active principle are not enough if it is trapped in its formulation or it is released erroneously. The absence of dissolution method can reduce the drug to the level of an adjuvant. Therefore, the objective of this study was to develop and validate a successful dissolution method for the evaluation of rifaximin tablets. The method contemplated the parameters for linearity, selectivity, precision, accuracy and robustness. It was found that for the dissolution of the tablets of rifaximin of 200 mg, paddle apparatus at 50 rpm and 900 mL of acetate buffer of pH 5.0 + 0.2 % SLS as dissolution medium are optimum conditions. The method presented is useful and can be applied for the routine quality control of tablets of rifaximin.

* Corresponding Authors
Fax: +55 16 3301 6967
E-Mail: ac_kogawa@yahoo.com.br
[a] Faculdade de Ciências Farmacêuticas de Araraquara, UNESP, Rodovia Araraquara-Jaú, km 1, CEP 14801-902, Araraquara, SP, Brazil.

Introduction

The dissolution in the gastrointestinal tract is the biggest challenge for solid oral dosage forms.[1-2] The main applications of the dissolution tests are in drug development, stability studies, establishment of in vitro - in vivo correlations (IVIVC) that can be used to reduce the number of human studies during the development of a particular formulation[3] and they can also be used as a substitute for bioequivalence tests,[4] quality control and pharmaceutical equivalence studies.

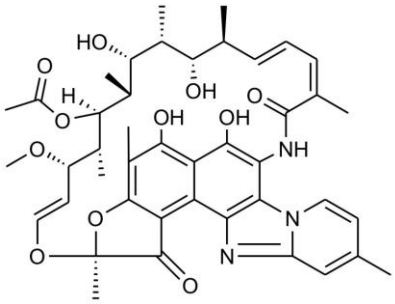

Figure 1. Chemical structure of rifaximin (CAS 80621-81-4).

For solid dosage form for immediate release of rifaximin, no method for dissolution has been described in official compendia[5-10] or literature. Rifaximin (Figure 1) is an oral antibiotic with broad spectrum of action that acts locally in the gastrointestinal tract. It reaches high concentrations in the human intestine, where it is active against many enteropathogens.[11-13] In market tablets of 200 mg are found. The dose is 600 mg (1 tablet 3 times daily) or 800 mg (2 tablets 2 times a day).

The development of effective analytical methods for the quality control of marketed drugs is extremely important and aims to provide reliable information about the nature and composition of the materials in question.[14] Validation is an important part of the quality assurance program and aims to demonstrate that the analytical method is suitable for the intended proposal and safe to run.[15]

Analytical methods for rifaximin, available in literature and official compendia for its quality control, lacked a method to evaluate the dissolution of tablets. In its absence, the drug does not have the quality required for its safe and effective use. A practical and accurate analysis method is a primary step in the rational use of pharmaceuticals.[16] Therefore, a dissolution method to tablets of rifaximin has been developed and validated in this work.

Experimental

The standard was rifaximin, content 99.0 %, acquired from NutraTech Development Limited (China). The pharmaceutical form was rifaximin tablets of 200 mg (labeled content), lot 12927, under the trade name Flonorm™, of the Laboratory Gonher Farmaceutica LTDA.

The placebo was prepared by physical mixing of excipients i.e., talc, microcrystalline cellulose, glycerol palmitostearate, titanium dioxide, colloidal anhydrous sílica, red iron oxide, disodium edetate, sodium carboxymethyl, hypromellose and propylene glycol.

Determination of the optimum dissolution conditions

For establishing optimum conditions, dissolution were tested in purified water, HCl 0.01 M, phosphate buffer of pH 6.2 (50 mM) + 0.5 % Tween 20, phosphate buffer of pH 6.2 (50 mM) + 0.5 % Tween 80 and phosphate buffer of pH 6.2 (50 mM) + 1.0 % sodium lauryl sulfate (SLS). In the first test, 200 mg of the rifaximin standard was added to 333 mL of dissolution medium under agitation at 37 ± 0.5 °C. In the

second test, the media chosen was one in the dissolution of the drug was to be tested. Others pH values, tested in the second test, were pH 5.0 and pH 6.8. 10 mg of rifaximin was added to tubes containing 2.5 mL of each medium evaluated and then they were submitted to agitation in a shaker at 60 rpm for 36 hours at 37 ± 0.5 °C. After the equilibrium time, the samples were filtered using quantitative filters and the absorbances were measured spectrophotometrically in the UV region.

Determination of the dissolution profiles

The dissolution profiles for rifaximin tablets of 200 mg were obtained using the various media viz., acetate buffer of pH 5.0 (50 mM) + 0.1 % SLS, acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS and acetate buffer of pH 5.0 (50 mM) + 0.5 % SLS. The media was deaerated by ultrasound for 30 min under 40 °C. The apparatus paddle under speeds of agitation of 50, 75 and 100 rpm was tested. The sampling times were 5, 10, 15, 20, 30 and 60 min. At the appointed times, 10 mL aliquots were taken from each sample and immediately filtered. The same volume of dissolution medium was replaced in order to maintain a constant total volume. The absorbances were measured spectrophotometrically in the UV region.

Validation of the method

The validation of the dissolution method was performed by evaluation of linearity, detection and quantification limits, selectivity, precision, accuracy and robustness.

Linearity

The linearity test was performed by creating a Ringbom curve by determining the absorbance of 29 concentrations of rifaximin standard, varying from 1 to 200 μg mL^{-1}. For this, a solution of 200 μg/mL of rifaximin standard was prepared in acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS.

From the Ringbom curve 6 points at concentrations from 15 to 50 μg mL^{-1} of rifaximin standard was chosen for evaluating the linearity of the method. The absorbance of the solutions were determined at 290 nm using acetate buffer pH 5.0 (50 mM) + 0.2 % SLS as blank. The analytical curve was constructed on three different days and in triplicate. The data obtained in the construction of the calibration curve were analyzed to obtain the equation of the line by the least squares method, and the check of linearity and parallelism was confirmed by Analysis of Variance (ANOVA).

Limit of detection (LD) and quantification (LQ)

LD of rifaximin was determined from the three calibration curves obtained, using the data of the standard deviation of the intercept (*s*) and average slope (*L*), according to Eqn. (1).

$$LD = (3.3s)/L \quad (1)$$

LQ of rifaximin was obtained with the same data described above, according to Eqn. (2).

$$LD = (10s)/L \quad (2)$$

Selectivity

The selectivity of the method was tested using placebo of rifaximin tablets. The placebo was prepared using amounts of excipients equivalent to one tablet of Flonorm™ 200 mg, which was transferred to dissolution cubas containing 900 mL of acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS. The placebo was submitted to dissolution test under the conditions of the paddle for 1 h at 150 rpm. The possible interference of adjuvants was analyzed spectrophotometrically in the UV region.

Precision and accuracy

The precision and accuracy were evaluated at levels of 50 %, 100 % and 150 % of the theoretical concentration of the test. To obtain rifaximin stock solution, a solution of 900 μg mL^{-1} in acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS was prepared. In order to obtain solutions at the level of 50 % of the theoretical concentration of the test, 15 mL of rifaximin stock solution were transferred to dissolution cubas containing 900 mL of acetate buffer pH 5.0 (50 mM) + 0.2 % SLS. Then, amounts of adjuvants equivalent to 50 % of the amount contained in one tablet Flonorm™ 200 mg was added to the cuba. In order to obtain solutions at the level of 100 % of the theoretical concentration of the test, 30 mL of rifaximin stock solution were transferred to dissolution cubas containing 900 mL of acetate buffer pH 5.0 (50 mM) + 0.2 % SLS. Then, amounts of adjuvants equivalent to 100 % of the amount contained in one tablet Flonorm™ 200 mg was added to the cuba. In order to obtain solutions at the level of 150 % of the theoretical concentration of the test, 45 mL of rifaximin stock solution were transferred to dissolution cubas containing 900 mL of acetate buffer pH 5.0 (50 mM) + 0.2 % SLS. Then, amounts of adjuvants equivalent to 150 % of the amount contained in one tablet Flonorm™ 200 mg was added to the cuba. The procedure was performed in triplicate. The solutions were subjected to dissolution test under the conditions of the paddle for 1 h at 50 rpm and analyzed spectrophotometrically in the UV region. On the analysis of the parameter precision, the relative standard deviation (RSD) was calculated for each level. This parameter was evaluated by intraday and interday precision. For the analysis of accuracy parameter, the percentage of recovery of rifaximin in each cuba was calculated by dividing the value obtained by the theoretical value corresponding to each concentration.

Robustness

The robustness was evaluated using the Youden and Steiner test.[17] 7 variables with the potential of interference in the analysis were selected. They are dissolution time, pH of the dissolution medium, speed of agitation, temperature of the dissolution medium, deaeration of the dissolution medium, filtration through membrane pore 0.45 μm and exposure to light.

The normal conditions of analysis were defined as A, B, C, D, E, F and G and the changes as a, b, c, d, e, f and g. Eight experiments were performed as indicated in Table 1.

Table 1. Conditions tested for the robustness parameter.

Condition	Normal	Changed	Experiments							
			1	2	3	4	5	6	7	8
Dissolution time	60	55	A	A	A	A	a	a	a	a
pH	5.0	4.8	B	B	b	b	B	B	b	b
Speed (rpm)	50	45	C	c	C	c	C	c	C	c
Temp. (°C)	37	35	D	D	d	d	d	d	D	D
Deaeration	yes	no	E	e	E	e	e	E	e	E
Filter	yes	no	F	f	f	F	F	f	f	F
Light	yes	no	G	g	g	G	g	G	G	g

From these results, the effect of each variable was estimated by the difference between the average of results of the four analyzes with the capital letter and the average of the results of the four analyzes with the lowercase letter. Considering the standard deviation of the eight results, values with the effect greater than the product of the standard deviation by the square root of two are considered significant and alter the analytical response.

Results

Determination of the Optimum Conditions

The results of the first dissolution tests of rifaximin for determination of the optimum conditions of the dissolution medium for the rifaximin tablets are shown in Table 2. The solubility of the drug was judged visually. The solubility test was performed in media which dissolved rifaximin and the results are shown in Table 3. The media to ensure optimum conditions for dissolution of rifaximin are acetate buffer of pH 5.0 (50 mM) + 0.5 % SLS, acetate buffer of pH 5.0 (50 mM) + 1.0 % SLS and phosphate buffer of pH 6.2 (50 mM) + 1.0 % Tween 20.

Table 2. Solubility of rifaximin tablets in different media.

Solvent	Results
Purified water	Insoluble
HCl 0.01 *M*	Insoluble
Phosphate buffer of pH 6.2 (50 mM) + 0.5 % Tween 20	Partially soluble
Phosphate buffer of pH 6.2 (50 mM) + 0.5 % Tween 80	Partially soluble
Phosphate buffer of pH 6.2 (50 mM) + 1.0 % SLS	Partially soluble

Determination of the dissolution profiles

The dissolution profile of rifaximin in acetate buffer of pH 5.0 (50 mM) + 0.1 % SLS, acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS and acetate buffer of pH 5.0 (50 mM) + 0.5 % SLS is shown in Figure 2. The samples were analyzed by spectrophotometrically at 290 nm.

Table 3. Solubility of rifaximin in different media.

Solvent	Concentration (μg mL^{-1})
A + 0.5 % Tween 20	360.38
A + 1.0 % Tween 20	362.94
A + 0.5 % Tween 80	185.10
A + 1.0 % Tween 80	430.32
A+ 0.5 % SLS	1792.16
A + 1.0 % SLS	2188.84
B + 0.5 % Tween 20	302.08
B + 1.0 % Tween 20	751.41
B + 0.5 % Tween 80	283.86
C + 0.5 % Tween 20	402.11
C + 0.5 % Tween 80	384.26

A = Acetate buffer of pH 5.0 (50 mM), B = Phosfate buffer of pH 6.2 (50 mM), C = Phosfate buffer of pH 6.8 (50 mM).

Table 4. ANOVA of the analytical curves of spectrophotometric determination of rifaximin in the UV region.

Variation sources	DF[a]	SS[b]	Varian-ce	F_{cal}	F_{tab} (0.05)[c]
Among concentrations	5	0.64	0.13	18.75*	3.11
Linear regression	1	0.64	0.64	93.70*	4.75
Linearity deviation	4	0.00	0.00	0.01	3.26
Inside (waste)	12	0.01	0.00	-	-
Total	17	0.64	-	-	-

[a]DF = degrees of freedom, [b]SS = sum of squares, [c]significant for p<5 %

The acetate buffer of solutions of pH 5.0 (50 mM) + 0.5 % SLS and acetate buffer of pH 5.0 (50 mM) + 0.1 % SLS are not suitable for tests and dissolution profiles for rifaximin tablets 200 mg. In the first medium, in 90 min, the dissolution was 90.55 % and in the second médium, in 60 min the dissolution was only 29.91 %. The acetate buffer of solutions of pH 5.0 (50 mM) + 0.2 % SLS is suitable for tests and dissolution profiles for rifaximin tablets 200 mg. In this medium, there was a release of 92.06 % of the drug in 60 min.

The influence of speed of agitation was tested to determine the optimum conditions of tests and dissolution profiles for rifaximin tablets 200 mg. A dissolution profile using tablets 200 mg of rifaximin, apparatus paddle at 50 and 75 rpm and acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS as dissolution medium was built and is shown in Figure 3. The speed of agitation of the paddles has an influence on the release of rifaximin. The only condition which allowed one point higher than 85 % of dissolution was speed 50 rpm, which allows the comparison of dissolution profiles by the method "Simple Model Independent."

The conditions that can be used to compare dissolution profiles of rifaximin by the method "Simple Model Independent" are apparatus paddle with 50 rpm, acetate buffer of pH 5.0 (50 mM) + 0.2 % SLS as dissolution medium. These conditions allow obtaining a discriminatory dissolution profile, which can be used in pharmaceutical equivalence studies and routine tests of quality control.

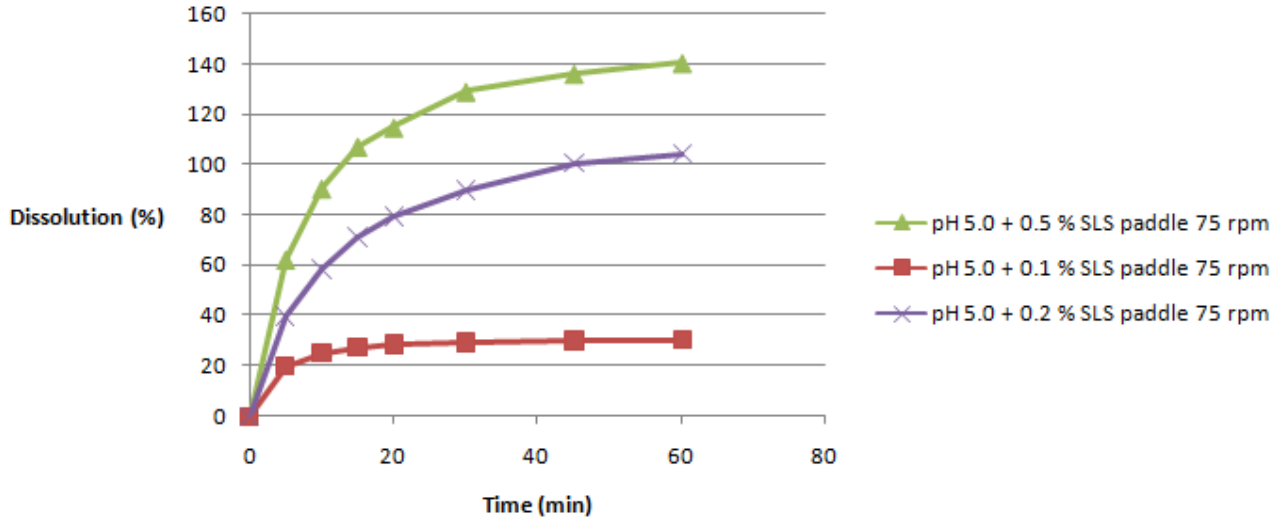

Figure 2. Dissolution profile of tablets containing 200 mg of rifaximin.

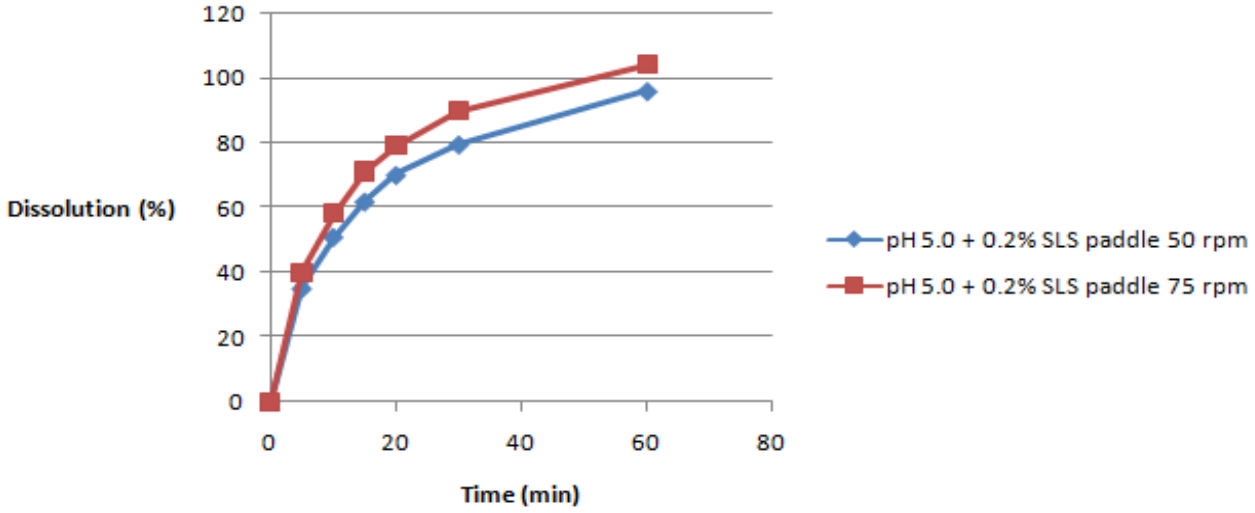

Figure 3. The dissolution profile of tablets containing 200 mg of rifaximin in acetate buffer of solution of pH 5.0 (50 mM) + 0.2 % SLS.

Table 5. Evaluation of the precision parameters for the dissolution test of rifaximin tablets 200 mg.

Level		Absorbances						Average	SD	RSD (%)
		1	2	3	4	5	6			
Intraday	50%	0.228	0.226	0.218				0.224	0.01	2.36
	100%	0.440	0.430	0.425				0.432	0.01	1.77
	150%	0.659	0.655	0.640				0.651	0.01	1.54
Interday	50%	0.209	0.200	0.198	0.228	0.226	0.218	0.213	0.01	6.04
	100%	0.406	0.385	0.394	0.440	0.430	0.425	0.413	0.02	5.25
	150%	0.612	0.578	0.570	0.659	0.655	0.640	0.619	0.04	6.24

Validation of the method

The analytical curve of rifaximin, for the linearity parameter, was built with the average of the absorbance values of three analytical curves with concentrations from 15 to 50 µg mL^{-1}, after evaluation of the results of Ringbom curve.

The equation of the line, determined by the method of least squares, is $y = 0.0150x + 0.0164$, with a correlation coefficient (r) equal to 0.9999 for the rifaximin standard. ANOVA calculated for the data of the analytical curves of the rifaximin standard is shown in Table 4. The results of limit of detection and limit of quantification are1.50 µg mL^{-1} and 4.53 µg mL^{-1}, respectively.

Figure 4 shows the spectrum concerning the evaluation of the adjuvants contained in the tablet Flonorm™ 200 mg in the parameter selectivity.

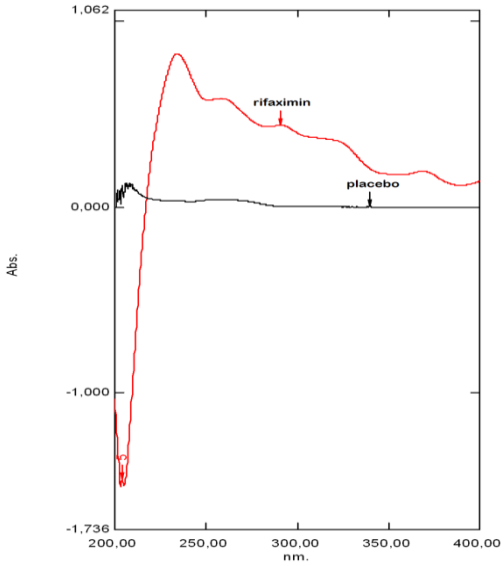

Figure 4. Overlapping of spectra of adjuvants of the tablet Flonorm™ 200 mg and rifaximin standard solution at a concentration of 30 µg mL^{-1} in acetate buffer of of pH 5.0 (50 m*M*) containing 0.2 % SLS.

The values obtained in the evaluation of precision of parameters and in the recovery test of rifaximin in the dissolution test are shown in Table 5 and 6, respectively. The values of percentage of rifaximin dissolved in experiments 1 to 8 are 96.04, 96.34, 100.79, 89.79, 95.54 and 88.89, respectively. The value of standard deviation multiplied by square root of 2 is 5.91. The effect of each condition as calculated from the values found is given in Table 7.

Table 6. Evaluation of the accuracy parameter for the dissolution test of rifaximin tablets 200 mg.

Level	Added µg mL^{-1}	Recovered µg mL^{-1}	Recovery %	RSD %
50 %	15	15.03	100.18	3.99
100 %	30	29.87	99.57	1.70
150 %	45	44.52	98.92	1.50

Table 7. Effects obtained according to the Youden and Steiner test.

Condition	Effect
Dissolution time	1.98
pH	2.00
Speed (rpm)	2.70
Temperature (°C)	-1.10
Deaeration	2.65
Filter	-6.13
Light	0.48

Discussion

In vitro dissolution tests have emerged as preferred method to evaluate the development of new active ingredients, pharmaceutical formulations and perform pharmaceutical equivalence studies.[7, 9-10, 15, 18-20]

The tablets of rifaximin have yet not been standardized by dissolution method. This status blinds the pharmaceutical product behavior that can have the best characteristics but is trapped in its formulation or is released erroneously in the organism. The absence of dissolution method for a pharmaceutical product can regress the action of the drug to that of an adjuvant.

In the comparison of dissolution profiles by the method “Simple Model Independent” just one point above 85 % dissolution must be included for product.[21] Thus, the conditions of 37.5 °C, paddle at 50 rpm and acetate buffer of pH 5.0 (50 mmol L^{-1}) with 0.2 % SLS have been chosen to allow the creation of a discriminatory dissolution profile, which can be used in pharmaceutical equivalence study of tablets of rifaximin 200 mg.

The presently developed method for dissolution test of tablets of rifaximin 200 mg presents satisfactory linearity in the range of 15 to 50 µg mL^{-1} with r = 0.9999, as recommended by the ICH.[15] The selectivity of the method has been proven by analyzing the spectrum of adjuvants solution of rifaximin tablets, which demonstrated that the excipients do not interfere in the absorbance in the UV region. The proposed method of the dissolution test is precise because estimates for RSD of less than 6.5 % are obtained in the interday and intraday precision.

This method led to a recovery between 95 and 105 % and as per the literature recommendation[10] is considered as accurate.

According to Youden and Steiner,[17] the variables that have a greater effect than the square root of two multiplied by the standard deviation between the results influence the analytical response significantly. Thus, the condition "no filter" showed influence on the analytical response to tablets of rifaximin 200 mg, since its effect was 6.13 and the square root of two multiplied by the standard deviation between the results was 5.91. This is a warning not to change this specific condition. "No filter" allows the permanence of particles in the solution which until then had not been dissolved and over time, previous to reading, they dissolve. This fact generates different results.

The method was robust towards the other changes made in dissolution time, pH of the medium, the rotational speed of the paddles, temperature, deaeration, and the presence or absence of light.

Conclusions

The dissolution test of tablets of rifaximin 200 mg has been performed successfully at the conditions of the paddle at 50 rpm, acetate buffer of pH 5.0 (50 mmol L^{-1}) with 0.2 % SLS and at 37.5 °C. The method proved to be reliable and can easily be used to evaluate the quality of rifaximin tablets by laboratories and pharmaceutical industries because it predicts its behavior in the organism.

Acknowledgements

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ANTIOXIDANTS: AN OVERVIEW ON THE NATURAL AND SYNTHETIC TYPES

Emad M. Atta[a,b]*, Nawal H. Mohamed[c,d] and Ahmed A. M. Abdelgawad[d]

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Antioxidants were used to prevent oxidation process in foods which lead to rancidity and browning, DNA oxidation and have many positive physiological effects in human. The concentration and the absorption mechanism of natural antioxidants are important in obtaining the maximum beneficial effect. The sources of antioxidants must be carefully considered to maximize absorption and avoid the toxicity of higher concentration of synthetic groups. A general lack of information about antioxidants indicates by a survey of the general public. An organized effort to educate individuals about foods rich in natural antioxidants and the ability to recognize the major synthetic antioxidants on food labels would be highly beneficial, though more research needs to be done to fully understand their physiological effects.

* Corresponding Authors
E-Mail: emadata@yahoo.com
[a] Chemistry Department, Faculty of Science, Jazan University, Jazan, KSA
[b] Genetic Engeneering. and Biotechnology Research Institute, University of Sadat City, Egypt
[c] Chemistry Department, Faculty of Science, Girls' Section, Jazan University, Jazan, KSA
[d] Aromatic and Medicinal Plants Department, Desert Research Center, Cairo, Egypt

Introduction

Antioxidants go through several processes before they can have consumed by the people. Researchers in the middle of the twenty-first century after doing many researches found that life span of people increases by the normal consumption of anti-oxidants and also it prevents several fatal diseases. At the end of 19th century, antioxidants are used for several industrial processes like prevention of metal corrosion, rubber vulcanization. The scientists found that these substances or anti-oxidants protects the metal from corrosion and limited the oxidation of the metals.[1-2]

Antioxidants are widely used as an ingredient in dietary supplement for promoting good health and preventing diseases like cancer, cardiovascular disease. In addition, they are also used as preservatives for foods. This indeed happened in the mid. 20th century. It all started with the attempts by scientists to extend the life of foods. By combining antioxidants with foods which have high unsaturated fat, the tests were able to prevent the onset of rancidity - a nasty process by which the unsaturated fats break down and produce a rancid-like smell and taste.[3] As this process continued; new information was brought to light, it was quickly discovered that a few of the key and vital vitamins - essential in the human diet – were actually able to be classified as antioxidants too. This meant that over the past 1000 years, people had been consuming antioxidants on a daily basis.[4]

The aim of this review is to explore the different types of natural and synthetic antioxidants, the suggested mechanism and physiological effects of each type as well, in addition to the potential health risks associated with consuming the synthetic antioxidants. On the other hand, an overview on the definitions, uses, popular methods to assay antioxidant activity was stated.

Oxidation of foods

Oxidation process

Most foods are made up of several organic compounds that can easily undergo oxidation. Lipids (such as fats, oils, and waxes) in general have the greatest tendency to lose electrons. Auto-oxidation of lipids in food Triggered by exposure to light, heat, ionizing radiation, metal ions or metallo-protein catalysts can have a deteriorating effect on the food colour, flavour, texture, quality, and safety. Fats contained in food are chemically composed of triglycerides and oxidation leading to the rancidity of foods occurs at the unsaturated sites of the triglycerides.[5]

Oxidants

The most common oxidants in biological systems are free radicals. Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable and active towards chemical reactions with other molecules. An unpaired electron in these free radicals, causes them to seek out and capture electrons from other substances in order to stabilize themselves. Although the initial attack causes the odd electron to be paired, another free radical is formed in the process, causing a chain reaction to occur.[6]

In the biological systems, the free radicals are often derived from oxygen, nitrogen and sulphur molecules. These free radicals are parts of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS). For example, ROS includes free radicals such as superoxide anion ($O_2^{-\bullet}$), perhydroxyl radical ($HO_2\bullet$), hydroxyl radical ($^{\cdot}OH$), nitric oxide and other species such as hydrogen peroxide(H_2O_2), singlet oxygen (O_2), hypochlorous acid (HOCl) and peroxynitrite

($ONOO^-$).[7] RNS are derived from nitric oxide through the reaction with $O_2^{-\bullet}$ to form $ONOO^-$. RSS are easily formed from thiols by reaction with ROS.[8] ROS are produced during cellular metabolism and functional activities.

Formation of free radicals in cells

Free radicals can be formed in 3 ways, (i) by homolytic cleavage of covalent bond of a normal molecule, with each fragment retaining one of the paired electrons, (ii) by loss of single electron from normal molecule and (iii) by addition of a single electron to a normal molecule. They are constantly being generated in vivo.[9]

Four endogenous sources appear to account for most of the oxidants produced by cells.

(i) Normal aerobic respiration in which mitochondria consume O_2, reduces it by sequential steps to produce O_2, H_2O_2, and $^{\bullet}OH$ as byproducts.

(ii) Bacteria or virus infected cells get destroyed by phagocytosis with an oxidative burst of nitric oxide (NO), O_2^-, H_2O_2 and OCl.

(iii) Peroxisomes produce H_2O_2 as a byproduct of fatty acid and other lipid molecular degradation, which is further degraded by catalase. Evidence suggests that, certain conditions favor escape of some of the peroxide from degradation, consequently releasing it into other compartments of the cell and increasing oxidative stress leading to DNA damage.

(iv) Animal Cytochrome P_{450} enzymes are one of the primary defense systems that provides protection against natural toxic chemicals from plants, the major source of dietary toxins. Even these enzymes are protective against acute toxic effects from foreign chemicals, yet they may generate some oxidative byproducts that damage DNA.[10-13]

Effect of oxidants on body tissue

Excessive amounts of free radicals can have deleterious effects on many molecules including protein, lipid, RNA and DNA since they are very small and highly reactive. ROS can attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, in which $^{\bullet}OH$ is the strongest oxidant. ROS attacking macromolecules is often termed oxidative stress. Cells are normally able to defend themselves against ROS damage through the use of intracellular enzymes to keep the homeostasis of ROS at a low level.

However, during times of environmental stress and cell dysfunction, ROS levels can increase dramatically, and cause significant cellular damage in the body. Thus, oxidative stress significantly contributes to the pathogenesis of inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, autism and aging.[14-17] In order to prevent or reduce the ROS induced oxidative damage, the human body and other organisms have developed an antioxidant defense system that includes enzymatic, metal chelating and free radical scavenging activities to neutralize these radicals after they have formed. In addition, intake of dietary antioxidants may help to maintain an adequate antioxidants status in the body.

Antioxidants

In foods, antioxidants have been defined as 'substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidisable materials such as fats,[18] therefore, in food science antioxidants are usually equated with chain-breaking inhibitors of lipid peroxidation, but not exclusively so. Many antioxidants have been studied and are used in a wide range of foods including beverages. Therefore, for foods and beverages, antioxidants are molecules that can be equated with the protection of macromolecules from oxidation.[19] In biological systems the accepted definition is that antioxidant is any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate.[20-21] This is a broader definition encompassing many vulnerable macromolecules (e.g. DNA, lipids and proteins) that can be affected by oxidation. In biological terms, it is accepted that any molecule that can retard or prevent the action of oxidants could be considered to be an antioxidant.[22] Such a broad definition means that compounds that inhibit specific oxidizing enzymes, react with oxidants before they damage molecules, sequester dangerous metal ions or even repair systems such as iron transport proteins, can fit the definition. Antioxidants can also be defined as substances that trap harmful forms of oxygen and prevent them from damaging cells.[23] Mechanistic definitions of antioxidants are usually focused on the ability to be a hydrogen donor or an electron donor. Many of the frequently cited assays of antioxidant capacity can be broadly categorized as either hydrogen transfer assays or single electron transfer reaction based assays. These assays measure the radical scavenging capacity or the reducing ability, respectively, not the preventative antioxidant capacity of the sample.[24]

Antioxidants process

Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. How do they work? The two possible pathways are chain-breaking and preventive.[25]

Chain-breaking: When a free radical release or abstracts an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs, either the radical is stabilized by a chain-breaking antioxidant such as β-carotene and vitamins C and E, or it simply decays into a harmless product.

Preventive: Antioxidant prevents oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron.

Mechanisms

If a compound inhibits the formation of free alkyl radicals in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the compound can delay the start or slow the chemical reaction rate of lipid oxidation. The initiation of free radical formation can be delayed by the use of metal chelating agents, singlet oxygen inhibitors and peroxide stabilizers. The propagation of free radical chain reaction can be minimized by the donation of hydrogen from the antioxidants and the metal chelating agents.[26]

(1) R:H + O::O + Initiator → R• + HOO•

(2) R• + O::O → ROO•

(3) ROO• + R:H → ROOH + R•

(4) RO:OH → RO• + HO•

(5) R::R + •OH → R:R-O•

(6) R• + R• → R:R

(7) R• + ROO• → ROOR

(8) ROO• + ROO• → ROOR + O_2

(9) ROO• + AH → ROOH + A•

(10) ROO• + A• → ROOA.

Scheme 1. Mechanism of antioxidants' action.

Antioxidant Defenses

Antioxidant means "against oxidation." Antioxidants work to protect lipids from per oxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gain the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken.[27] After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food which humans eat such as fruits, vegetables, seeds, nuts, meats, and oil.

Types of Antioxidants

Antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods. Dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, present in vegetables contribute both to the first and second defense lines against oxidative stress.[28]

Natural antioxidants

Natural antioxidant system is sorted in two major groups, enzymatic and non- enzymatic.

Non-enzymatic antioxidants: Non-enzymatic antioxidants include direct acting antioxidants, which are extremely important in defense against oxidation stress. Most of them, including ascorbic and lipoic acid, polyphenols and carotenoids, are derived from dietary sources. The cell itself synthesizes a minority of these molecules. Indirectly acting antioxidants mostly include chelating agents and bind to redoxmetals to prevent free radical generation.[29]

Vitamin E is a generic description for all tocopherol (Toc) and tocotrienol (Toc-3) derivatives. Tocopherols have a phytyl chain, while tocotrienols have a similar chain but with three double bonds at positions 3',7' and 11'. Both tocopherols and tocotrienols have four isomers, designated as α-, β-, γ- and δ-, which differ by the number and position of methyl groups on the chroman ring.[30] All of these molecules possess antioxidant activity, although α-tocopherol (α-Toc) is chemically and biologically the most active.[31-32] α-Tocopherol is the major vitamin E *in vivo* and exerts the highest biological activity. Tocopherols are present in polyunsaturated vegetable oils and in the germ of cereal seeds, whereas tocotrienols are found in the aleurone and subaleurone layers of cereal seeds and in palm oils.

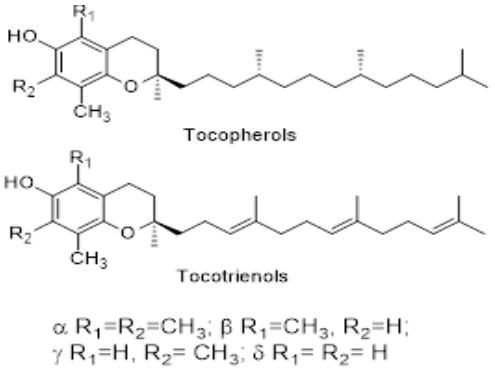


Figure 1. Structure of vitamin E.

It has been reported that α-Toc-3 possessed 40- to 60-fold higher antioxidant activity than α-Toc against ferrous iron/ascorbate- and ferrous iron/NADPH-induced lipid peroxidation in rat liver microsomes[33] and that α-Toc-3 exhibited greater peroxyl radical scavenging potency than α-Toc in liposomal membranes.[34]

The antioxidant reaction of *α*-tocopherol is not a reaction with oxygen. Many molecules react with oxygen, but they do so without being antioxidants. β-Carotene, for example,

readily reacts with oxygen, but it is by no means an efficient antioxidant.[35] The basis of an antioxidant reaction is not the removal of oxygen but the interception of the autoxidation radical chain process which is not perpetuated by oxygen but by the fatty acid.

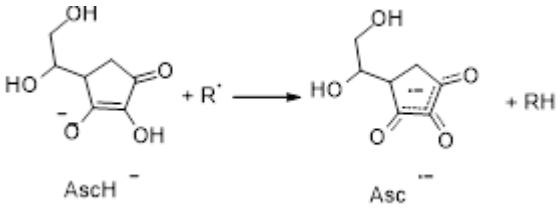
α-Tocopherol reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation, and intercepts the chain reaction.[36] What makes α-tocopherol such a highly efficient antioxidant is (i) that it reacts with the peroxyl radical extremely fast, much faster than to allow for the peroxyl radical to do any other reactions; (ii) it takes away the radical character from the oxidizing fatty acid and prevents it from further radical reactions; (iii) in the antioxidant reaction, α-tocopherol is turned into a fairly stable radical. Under normal circumstances, it will only react with another radical (either a tocopheroxyl radical or a fatty acid peroxyl radical) to form stable, non-radical products. In this setting, α-tocopherol is the most powerful lipid soluble antioxidant known, and only recently novel synthetic antioxidants have been developed that surpass α-tocopherol's antioxidant capacity.[37] Chemically, abstraction of the 6-OH hydrogen yields a tocopheroxyl radical. Tocopherol can be restored by reduction of the tocopheroxyl radical with redox-active reagents like vitamin C (ascorbate) or ubiquinol.[38-42] In homogeneous solution phase autoxidation, the tocopheroxyl radical will react with a second peroxyl radical to give non radical products. This second reaction leads to the destruction of a tocopherol as an antioxidant. Thus, one molecule of a-tocopherol can terminate two autoxidation chains.

The main source for dietary uptake of vitamin E is plant food (vegetables, fruits, seeds, and seed oils). Sunflower seeds, olive oil, and almonds are rich sources of α-tocopherol. While other seeds and seed oils generally contain more γ-tocopherol than α-tocopherol, the opposite is true for green leaves. β-Tocopherol and δ-tocopherol are the least abundant, and so, in general, are the different tocotrienols.[43]

Vitamin C (ascorbic acid and ascorbate) is a six-carbon lactone which is synthesized from glucose by many animals. Vitamin C is a water-soluble vitamin. As such, it scavenges free radicals that are in an aqueous (water). When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy.[44]

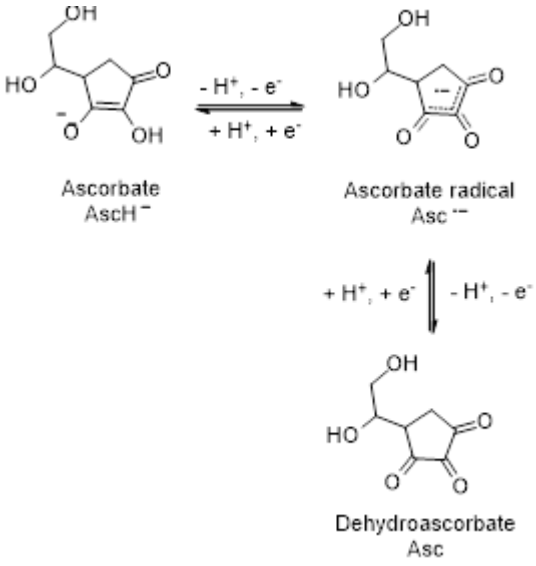
Vitamin C is an electron donor (reducing agent or antioxidant), and probably all of its biochemical and molecular functions can be accounted for by this function. Vitamin C acts as an electron donor for 11 enzymes.[45-46] Gastric juice vitamin C may prevent the formation of *N*-nitroso compounds, which are potentially mutagenic.[47] High intakes of vitamin C correlate with reduced gastric cancer risk,[48] but a cause and effect relationship has not been established. Vitamin C protects low-density lipoproteins ex *vivo* against oxidation and may function similarly in the blood.[49]

Vitamin C plays an important role in the production of collagen. Collagen gives your skin elastic properties. As people get older, their skin contains lower levels of collagen. Most anti-aging creams, therefore, include plenty of Vitamin C. This keeps the skin young and healthy by improving elasticity.



Scheme 2. Formation of ascorbate radical.

A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilize folate in food and in plasma, and increased excretion of oxidized folate derivatives in human scurvy was reported.[50] Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous, Fe^{2+}) form.[51-52] The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends largely on the amount of inhibitors, such as phytates and polyphenols, present in the meal.[53] Vitamin C (ascorbate, $AscH^-$), for example, can donate a hydrogen atom to a free radical molecule thereby neutralizing the free radical **(**ascorbic acid, generally acts as an antioxidant by donating hydrogen atoms from its own hydroxyl groups in order to quench reactive radical species) and generating double bonds in place of the lost hydrogen to make up for the lost electron density. However, once this occurs, the Vitamin C molecule itself is oxidized, and so it is reduced back into a useable form of the Vitamin C molecule by a variety of enzymes, including glutathione.



Scheme 3. Oxidation of Vitamin C.

But the ascorbic acid free radical is very stable because of its resonance structure.[54] In general, recent literature on the interaction between vitamin C and vitamin E has provided strong support for the non-enzymatic regeneration of α-tocopherol from the α-tocopheroxyl radical, formed when α-tocopherol scavenges a peroxyl radical (ROO•), by ascorbic acid.

Vitamin C is found in many fruits and vegetables.[55] Citrus fruits and juices are particular important sources of vitamin C but other fruits including cantaloupe, honeydew melon,

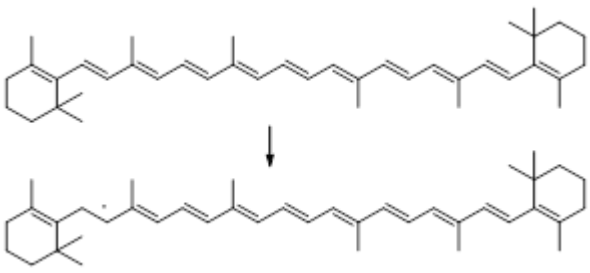
cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, watermelon, and tomatoes also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, Brussels sprouts, beansprouts, cauliflower, kale, mustard greens, red and green peppers, peas, tomatoes, and potatoes may be more important sources of vitamin C than fruits. This is particularly true because the vegetable supply often extends for longer periods during the year than does the fruit supply.

β-Carotene

Carotenoids are a widespread group of naturally occurring fat-soluble colorants. In developed countries, 80-90% of the carotenoid intake comes from fruit and vegetable consumption. Of the more than 700 naturally occurring carotenoids identified, only six of them (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin) account for more than 95% of total blood carotenoids. β-Carotene (BC) is a naturally occurring orange-colored carbon-hydrogen carotenoid, abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables.[56] It is also the most widely distributed carotenoid in foods.[57] BC undergoes trans (E) to cis (Z) isomerization,[58] whereas the (all-E)-form is the predominant isomer found in unprocessed carotene rich plant foods.[59-60]

Nutrition has a significant role in the prevention of many chronic diseases such as cardiovascular diseases (CVD), cancers, and degenerative brain diseases.[61] The consumption of food-based antioxidants like BC seems to be useful for the prevention of macular degeneration and cataracts.[62] It is also available in synthetic forms and is commercially processed from substances such as palm oil and algae. BC has potential antioxidant biological properties due to its chemical structure and interaction with biological membranes.[63] It is well-known, that BC quenches singlet oxygen with a multiple higher efficiency than α-tocopherol.[64] In addition, it was shown that (Z)-isomers of BC possess antioxidant activity in vitro.[65-67]

The other strategy with which antioxidants prevent oxidation is to use double bonds to donate electron density. As the electrons in double bonds are less tightly held to the molecule, they are more easily available for donation. Generally, antioxidants that use this strategy are non-polar, and contain hydrocarbon chains of moderate length. Three prominent natural antioxidants that utilize this method are carotene, lycopene, and vitamin A. Carotene and lycopene have very similar mechanisms of antioxidant activity, as both have similar chemical structures and fall into the carotenoid family of molecules.



Scheme 4. Oxidation of carotene via the donation of an electron from a double bond.

Phenolic antioxidants

Phenolic compounds are a large group of the secondary metabolites widespread in plant kingdom. They are categorized into classes depending on their structure and subcategorized within each class according to the number and position of hydroxyl group and the presence of other substituents. The most widespread and diverse group of the polyphenols are the flavonoids which are built upon C6-C3-C6 flavone skeleton. In addition, other phenolic compounds such as benzoic acid or cinnamic acid derivatives have been identified in fruits and vegetables.[68-69]

Phenolic compounds, especially flavonoids, possess different biological activities, but the most important are antioxidant activity, capillary protective effect, and inhibitory effect elicited in various stages of tumor.[70-73] Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as number and location of hydroxyl groups. In many in vitro studies, phenolic compounds demonstrated higher antioxidant activity than vitamins and carotenoids.[74-75]

The major antioxidative phenolics in plants can be divided into four general groups viz., phenolic acids (gallic, protochatechuic, caffeic, and rosmarinic acids), diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin), and volatile oils (eugenol, carvacrol, thymol, and menthol).[76] Phenolic acids generally act as antioxidants by trapping free radicals whereas flavonoids can scavenge free radicals and metal chelates as well.[77]

Many mechanisms have been proposed for polyphenol prevention of oxidative stress and ROS/RNS generation both *in vitro* and *in vivo*. Radical scavenging by polyphenols is the most widely published mechanism for their antioxidant activity. In this radical scavenging mechanism, polyphenols sacrificially reduce ROS/RNS, such as $^{\bullet}OH$, $O_2^{\bullet-}$, $NO^{\bullet}$, or $OONO^-$ after generation, preventing damage to biomolecules or formation of more reactive ROS.[78-80] The spatial arrangement of substituents is perhaps a greater determinant of antioxidant activity than the flavone backbone alone. Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity.[81-83]

Selenium

Selenium (Se) is an essential trace element and its deficiency in humans has been linked to increased risk of various diseases, such as cancer and heart diseases. Good food sources of selenium include fish, shellfish, red meat, grains, eggs and chicken. Vegetables can also be a good source if grown in selenium-rich soils. This mineral is thought to help fight cell damage by oxygen-derived compounds and thus may help protect against cancer. It is best to get selenium through foods, as large doses of the supplement form can be toxic. The level of Se generally depends on its level in soil.[84] Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. Since its discovery as an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxinreductase (TrxR) and

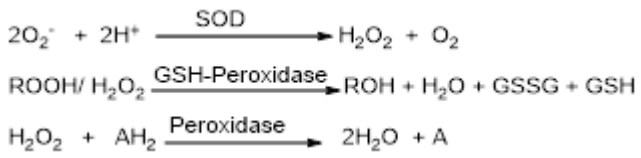
iodothyroninedeiodinases (IDD), there has been an increased interest in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes).[85]

The selenoenzymes that are found to have strong antioxidant activity include six groups of the GPx-GPx1, GPx3, GPx4, GPx5 and GPx6. These GPx play a significant role in protecting cells against oxidative damage from ROS and RNS, which include superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide and peroxynitrite.[86-87] The other essential antioxidant selenoenzymes are the TrxR where they use thioredoxin (Trx) as a substrate to maintain a Trx/TrxR system in a reduced state for removal of harmful hydrogen peroxide.[88-89] There are three types of TrxR that have been identified, and these include cytosolic TrxR1, mitochondrial TrxR2 and spermatozoa-specific TrxR, (SpTrxR).[90-91] Increasing evidence suggests that selenoprotein may also play a significant role in antioxidant defense system in preventing attack from harmful ROS and RNS.[92-93]

Enzymatic antioxidants

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more palpable.[94] Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health.

The repair enzymes that can recreate some antioxidants are SOD, GPx, glutathione reductase (GR), CAT and the other metalloenzymes. SOD, CAT, and GPx constitute a mutually supportive team of defense against ROS. While SOD lowers the steady-state level of O^{2-}, catalase and peroxidases do the same for H_2O_2.



Scheme 5. Mechanism of action of enzymatic antioxidant.

The antioxidant enzymes–GPx, heme peroxidase, CAT, and SOD–metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity.[95]

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) serve as the primary line of defence in destroying free radicals. Glutathione plays several roles in the body e.g., it improves the effectiveness of Vitamin C. SOD first reduces (adds an electron to) the radical superoxide (O_2^-) to form hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase and GPx then work simultaneously with the protein glutathione to reduce hydrogen peroxide and ultimately produce water (H_2O). The oxidized glutathione is then reduced by another antioxidant enzyme glutathione reductase. Together, they repair oxidized DNA, degrade oxidized protein, and destroy oxidized lipids (fat-like substances that are a constituent of cell membranes). Various other enzymes act as a secondary antioxidant defense mechanism to protect from further damage.[95]

SOD is the antioxidant enzyme that catalyzed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPx reactions.[96-98] In humans, there are three forms of SOD, cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD).[99-100] SOD destroys O^{2-} by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates.[101] All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe, Mn or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N_3^- , CN^-,[102] and by F^-.[103]

SOD is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts. Among the common natural sources of SOD are cabbage, Brussels sprouts, wheat grass, barley grass and broccoli.

Catalase (CAT) is an enzyme responsible for the degradation of hydrogen peroxide. It is a protective enzyme present in nearly all animal cells. The functions of Human erythrocyte catalase include catalyzing the decomposition of H_2O_2 to water and oxygen. It is a tetramer of 4 polypeptide chains. As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes.[104]

Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The GPx contains a single selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity.[105] There are five GPx isoenzymes found in mammals. The function of glutathione peroxidase, therefore, is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free H_2O_2 to water. Levels of GPx in the body are closely linked with that of glutathione, the master antioxidant. Glutathione (GSH for short) is a tripeptide that not only protects the cells against ill effects of pollution; it is also acts as the body's immune system boosters. It is present in high concentrations in the cells and plays a pivotal role in maintaining them in reduced state lest they suffer damage by oxidation by free radicals.

Combination of certain antioxidants like glutathione, vitamin C and E, selenium and glutathione peroxidase are very powerful in helping the body fight against the free radicals. GSH ensures that the red blood cells remain intact and protect the white blood cells (which are responsible for immunity). Glutathione is found in vegetables and fruit, but cooking will significantly reduce its potency. Taking it as a supplement is a good idea.

Glutathione-S-transferase (GST) is a family of enzymes comprising a list of cytosolic, mitochondrial and microsomal proteins which are capable of multiple reactions with multitude of substrates, both endogenous or xenobiotic. GSTs contribute to the phase II biotransformation of xenobiotics by conjugating these compounds with reduced glutathione to facilitate dissolution in aqueous cellular and extra cellular media and from there, out of the body. GSTs catalyse conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids as well as metabolism of xenobiotics.

Glutathione reductase (GR) plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate levels of intracellular glutathione (GSH). GSH, in conjunction with the enzyme glutathione peroxidase (GP), is the acting reductant responsible for minimizing harmful hydrogen peroxide cellular levels. The regeneration of GSH is catalyzed by GR. GR catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione, using β-nicotinamide dinucleotide phosphate (NADPH) as the hydrogen donor. Molecules such as NADPH act as hydride donors in a variety of enzymatic processes. NADPH has been suggested to also act as an indirectly operating antioxidant, given its role in the re-reduction of GSSG to GSH and thus maintaining the antioxidative power of glutathione.

Chemical pathways of natural antioxidants

After absorption, all antioxidants undergo certain chemical reactions in order to protect other compounds from oxidation. Most natural antioxidants have areas of high electron density within themselves in order to prevent other molecules from remaining as radicals for extended periods of time.

Natural antioxidants donate electrons from two major electron-rich sources: hydroxyl groups and double bonds. After donating electrons, natural antioxidants undergo additional chemical reactions in order to facilitate their breakdown. The first major method that several antioxidants use in order to prevent oxidation in other compounds is to donate electrons from their hydroxyl (-OH) groups.[106]

Sources of natural antioxidants

Various antioxidants are supplied to human body through diet, both vegetarian as well as non-vegetarian. Vitamins C and E, β-carotene and coenzyme Q are the most common antioxidants of diet, out of which, Vitamin E is present in vegetable oils and found abundantly in wheat germ. It is fat soluble vitamin, absorbed in the gut and carried in the plasma bylipoproteins. Out if 8 natural state isomeric forms of vitamin E, α-tocopherol is the most common and potent isomeric form. Being lipid soluble, vitamin E can effectively prevent lipid peroxidation of plasma membrane.[107-108]

Plants (fruits, vegetables, medicinal herbs) may contain awide variety of free radical scavenging molecules such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins etc.), nitrogen compounds (alkaloids, amines, betalains etc.), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant activity.[109-112]

Synthetic antioxidants

Synthetic antioxidants are chemically synthesized compounds since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. Due to the inherent instability of natural antioxidants, several synthetic antioxidants have been used to stabilize fats and oils. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were originally developed to protect petroleum from oxidative gumming.[113] However, these compounds have been used as antioxidants in human foods since 1954 and are perhaps the most common antioxidants used in those foods today.[114] BHT and BHA not only have similar names, but similar structures and antioxidant activity and are often used together in fats and oils. Despite the fact that both BHT and BHA are included in the list of substances that are "generally accepted as safe". Certain chronic toxicity studies have implicated BHT as potential tumor promoter when fed at high levels.[115-116] In contrast, BHA and BHT, may both be important inhibitors of carcinogenesis, probably by way of their antioxidant function.[117] Thus, there have been some attempts to remove these antioxidants, TBHQ (tert-butylhydroxyquinone) is another synthetic antioxidant which is widely used in the feed industry. Like BHT and BHA, TBHQ has a benzene ring or phenol structure. Other examples of synthetic antioxidants are propyl gallate (PG), dodecyl gallate (DG), octylgallate (OG) and ethylene diaminetetraacetic acid (EDTA).

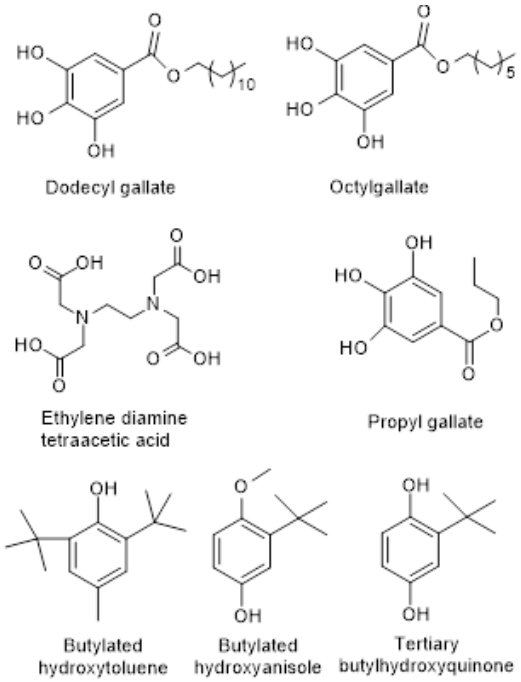


Figure 2. Structures of some synthetic antioxidant

Methods for assessing in vitro antioxidant activity

Due to the increasing interest in these biological molecules for consumers, food scientists and the medical fraternity, a quick and easy method for determining antioxidant capacity would be most useful. The most promising methods used to evaluate antioxidant properties were summarized by Krishnaiah et al.[118]

Uses of antioxidants in technology

Epidemiological studies have been reported that many of antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities to greater or lesser extent.[119]

In many cases, increased oxidative stress is a widely associated in the development and progression of diabetes and its complications which are usually accompanied by increased production of free radicals or failure of antioxidant defense.[120] Though the intake of natural antioxidants has been reported to reduce risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging, there is considerable controversy in this area.[121]

Leukocytes and other phagocyte destroy bacteria, parasites and virus-infected cells with NO, O_2, H_2O_2, and OCl^-, which are powerful oxidants and protect humans from infection. However, they also cause oxidative damage and mutation to DNA and participate in the carcinogenic process if remain unchecked.

It has been reported that antioxidants modulate the pathophysiology of chronic inflammation up to some extent.[122] Moreover, experiments and studies infer that antioxidants are needed to scavenge and prevent the formation of ROS and RNS, out of them, some are free radicals while some are not.[123] There is growing evidence that oxidative damage to sperm DNA is increased when there is vitamin C insufficiency in diet.[10] This strongly suggests the protective role of antioxidant in our daily diet.[124]

The antioxidants control gene behaviour and prevent diseases. The antioxidant network is body's built-in intelligence. It constantly monitors the health of each of the trillions of cells in your body. Whenever a problem is detected, antioxidants will turn on the appropriate gene, which, in turn, activates the cells that it needs to solve the problem. For example, antioxidants direct genes to alert the immune system when there are invading viruses are detected. The immune system then creates more white blood cells to kill the viruses. But the process begins with the antioxidant network. Because antioxidants can help regulate dangerous genes, it opens up the possibility to treat diseases at their root cause, by suppressing bad genes before they can do harm, using antioxidants-the ultimate preventive medicine.

Food Preservatives

Antioxidants are used as food additives to help guard against food deterioration.[125] Consequently, packaging of fresh fruits and vegetables contains an ~8% oxygen atmosphere milk and milk products like cheese; meat, fish and their products; spices and other dry foods like sugar, honey, beverages, and chewing gum.[126] Besides the direct addition to food items, the antioxidants can be used to preserve food by preventing the degradation of food packaging during processing and storage. Thus, antioxidants can be added to packaging materials like paper, polyethylene, plastic and paperboard preventing the oxidation of the material itself, or allowing the added antioxidants to migrate into the packaged food inside and prevent oxidation there.[127-128]

Antioxidants are an especially important class of preservatives.[129] These preservatives include natural antioxidants such as ascorbic acid and tocopherols as well as synthetic antioxidants such as, t-butylhydroquinone, BHA and BHT.[130-132] Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity.

Industrial uses

Antioxidants are frequently added to industrial products. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials. Polymers containing double bonds in their main chains, such as natural rubber are especially susceptible to oxidation and ozonolysis. Oxidation and UV degradation are also frequently linked, mainly because UV radiation creates free radicals by bond breakage. The free radicals then react with oxygen to produce peroxy radicals which cause yet further damage, often in a chain reaction.

Health benefits and risks

Due to the power of natural antioxidants to prevent the generation of free radicals, it has been found that they are particularly useful in preventing certain diseases. However, though it is apparent that natural antioxidants have many positive effects on health, it should also be taken into consideration that they could also have harmful effects if taken in excess.

Conclusions

In foods that may undergo oxidation, antioxidants, function as an inhibitor of oxidation reactions through various mechanisms. Nevertheless, some foods are deficient in natural antioxidants and can easily deteriorate during processing or in storage, necessitating the use of synthetic antioxidants. However, most synthetic antioxidants are effective at low concentrations, and the addition of higher levels may lead to a pro-oxidant effect. Additionally, large doses of synthetic antioxidants have been reported to impart safety problems.

Therefore, caution must be taken when selecting and adding antioxidants in food systems. Meanwhile, the safety of natural antioxidants should not be taken for granted as antioxidants from natural sources are attracting more and more attention.

The best way to get a variety of antioxidants in the diet is to eat foods that represent all the colours of the rainbow. Each color provides its own unique antioxidant effects. Bright orange, deep yellow fruits and vegetables like carrots, sweet potatoes, and apricots provide one type of antioxidant. Red foods like tomatoes provide another. Green vegetables, such as broccoli and cabbage, and blue or purple foods, like blueberries, each have their own antioxidant packages.

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ORIGIN OF ELECTRONIC PROPERTIES OF α-$Hg_3S_2Cl_2$ POLYMORPH

O. V. Bokotey[a]*, V. V. Vakulchak[a] and O. O. Bokotey[b]

Keywords: corderoite structure; DFT; energy dispersion; band gap.

To investigate the energy bands structure of α-$Hg_3S_2Cl_2$, first-principles calculations were performed within the density functional theory (DFT) formalism using the SIESTA software package. Using the local density approximation the atomic positions are relaxed so as to minimize the forces acting on the atoms. The analysis of band energy dispersion shows that the VBM and CBM are located at Γ symmetry point, resulting in a direct energy band gap of 3.198 eV. It is established that the nature of interatomic interactions has complex character and includes covalent and ionic parts. The inclusion of spin-orbit coupling does not strongly modifies the structure of energy bands.

* Corresponding Authors
E-Mail: bokotey_ov@ukr.net
[a] Faculty of Physics, Uzhhorod National University, 46 Pidgirna Str., Uzhhorod, 88000, Ukraine.
[b] Uzhhorod Specialized Boarding School, 28 Zagorska Str., Uzhhorod, 88000, Ukraine

Introduction

$Hg_3X_2Y_2$ (X = S, Se, Te; Y = F, Cl, Br, I) compounds present a class of perspective materials for nonlinear optics. Physical and chemical properties of these crystals are very useful for the creation of functional elements for optical devices. The corderoite family compounds are characterized by such optical properties as high refractive index and photoconductivity[1-3] and are materials showing presence of optical activity and electro-optic effect.[4-10] The main structural feature of mentioned crystals is the tendency to formation of various polymorphic modifications. At the same time due to the wide transparency in the visible and IR-range (from 0.3 to 40 μm) their optoelectronic features are very useful for possible applications.

Regarding α-$Hg_3S_2Cl_2$, no reports concerning electronic structure can be found in literature. Therefore, we thought it would be worthwhile to perform the band structure calculations of α-$Hg_3S_2Cl_2$. In this paper, the band structure and optical transitions of α-$Hg_3S_2Cl_2$ have been investigated using DFT. The study on the origin of electronic properties provides an important information and prediction of physical properties of title crystal.

Structure of α-$Hg_3S_2Cl_2$

The α-$Hg_3S_2Cl_2$ is cubic (a= 0.89 nm) and belongs to the space symmetry group T^5-$I2_13$. Figure 1 displays the crystal structure of the title compound. Observe that the basic structural unit in α-$Hg_3S_2Cl_2$ is a [SHg_3] pyramid. Crystal structure of gyrotropic α-$Hg_3S_2Cl_2$ was determined independently by different authors.[11-19] A characteristic structural feature is the presence of two sets of octahedral spirals with different radii and twisting directions. They are located side by side, oriented in the same direction and consistently alternated.[4]

There are three crystalline phases of $Hg_3S_2Cl_2$. These are corderoite minerals α-$Hg_3S_2Cl_2$ ($I2_13$), β-$Hg_3S_2Cl_2$ (Pm3n) and γ-$Hg_3S_2Cl_2$ (Pbmm),[20,21] which have variable structure depending on number of halogens entered into their structure. The first crystalline polymorph is metastable at normal conditions. The structure of the α-$Hg_3S_2Cl_2$ type is realized in the cases, when the chalcogen anion size is more than the halogen anion size: S^{2-}(0.182 nm), Se^{2-}(0.193 nm), Te^{2-}(0.211 nm) > Cl^-(0.181 nm); Te^{2-}(0.211 nm) > Br^- (0.196 nm). The main feature of this structural type is existence of $(Hg_3X_2)_{2n}^{2+}$ infinite chains from [XHg_3] trigonal pyramids connected by the mercury atoms which are joined for two pyramids. These chains unite in three-dimensional framework in which octahedral emptiness the halogen ions are localized.[3] The mercury atoms are two-coordinated with a linear geometry. The chalcogen atoms are three-coordinated, [SHg_3], forming trigonal pyramids. In title crystal, the interior bond angles Hg-S-Hg are 92° and the interior bond distances Hg-S and Hg-Cl are 0.245 and 0.292 nm. In this compound the lead atoms located at the 12b and 8a Wyckoff positions in the body-cantered unit cell. Due to specific atomic structure of corderoite-type compounds one can expect a possible applications of them as a non-linear optical materials.

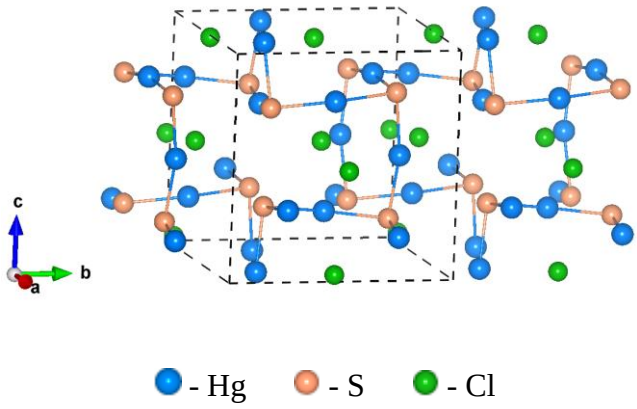

Figure 1. Crystal structure of α-$Hg_3S_2Cl_2$.

Calculation procedure

To calculate the energy bands structure of α-$Hg_3S_2Cl_2$, the DFT[22,23] with the local density approximation is used. The total and partial densities of electron states were determined by the tetrahedral method. We used the experimental lattice constants and atomic structure for these calculations. The DFT calculations are carried out on the minimized structure of α-$Hg_3S_2Cl_2$. Integration over the irreducible part of the Brillouin zone was conducted using the method of special k-points.[24,25] We determined that 4x4x4 k-point grid and a cut-off energy of 1200 Ry are sufficiently converged. We first relaxed the crystal structure with respect to the lattice constants and internal parameters. A Hubbard correction was used to obtain a better band gap value[26-28] that is mostly underestimated by LDA.

Results and Discussion

The calculated band structure of α-$Hg_3S_2Cl_2$ using LDA+U is presented in Figure 2. The energy structure of crystal under investigation defines by short-range order in the location of atoms (pyramids [SHg_3] and octahedras [HgS_2Cl_4]). Moreover, the indirect band gap indicated in the LDA calculation is changed to a direct band gap of 3.198 eV between the valence band maximum and conductive band minimum at Γ in the LDA+U calculation. It is in satisfactory agreement with experimental data from studies of diffuse reflectance spectra.[17] α-$Hg_3S_2Cl_2$ is a direct band gap semiconductor.

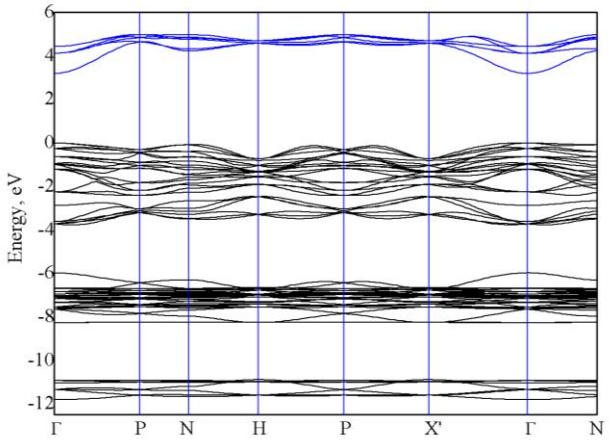

Figure 2. Band structure of α-$Hg_3S_2Cl_2$ polymorph

The partial DOS helps to identify the origin of electronic properties of α-$Hg_3S_2Cl_2$. The calculated partial density of states is illustrated in figure 3. The Fermi level is taken to be 0.0 eV. We have found that the conductive band is formed prevailingly from S-p/d states and Hg-s states. The valence band maximum consists of the S-p/Cl-p orbitals and a relatively small contribution of the Hg-s orbitals. Considering the DOS/PDOS, one can conclude that in α-$Hg_3S_2Cl_2$ the sulphur and chlorine atoms play more prominent role in the valence band than the mercury atoms, which is attributed to the higher electron negativity of sulphur and chlorine atoms than the mercury.

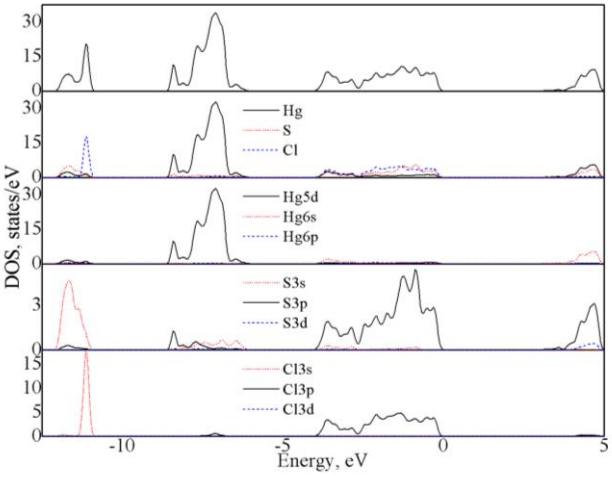

Figure 3. DOS/PDOS diagrams calculated for α-$Hg_3S_2Cl_2$.

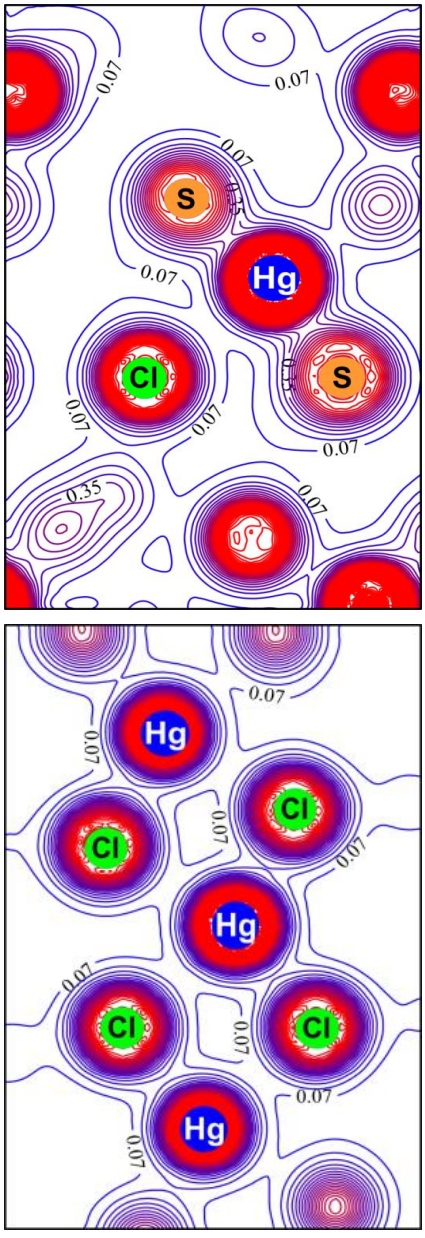

Figure 4. Spartial distribution maps of the charge density in α-$Hg_3S_2Cl_2$.

The bonding wave functions are localized around of chalcogen and halogen atoms.

The calculated DOS/PDOS of α-$Hg_3S_2Cl_2$ are compared with our previous DFT calculations for $Hg_3Se_2Cl_2$ and $Hg_3Te_2Cl_2$.[7,8] In general, the whole region of valence states has a complex hybrid character caused by the interaction of all atoms species inherent to the crystals of the $Hg_3X_2Y_2$ type. The analysis of rotation dispersion[16] of α-$Hg_3S_2Cl_2$ and our DFT calculations indicates that the rotation of polarization plane in this crystal is connected with direct optical transitions. Atomic transitions of [$Hg_3S_2Cl_4$] octahedra in excited electronic states makes contributions to gyrotropy caused by C_1 local symmetry group.[4] It should be noted that the direct optical transitions in the α-$Hg_3S_2Cl_2$ are localized on [$Hg_3S_2Cl_4$] octahedras.

The investigation of the band structure of solids including rather heavy atoms requires including the effect of spin-orbit coupling. The spin-orbit splitting in mercury orbitals are slight at the conductive band minimum. Thus, it not significantly affects the band gap size. In addition, the results from the calculation using spin-orbit coupling shows that the band topology near the conductive band minimum is insignificantly changed due to a larger spin-orbit splitting in the S-p orbitals at the Γ point.

To understand the bond nature of Hg-S and Hg-Cl in α-$Hg_3S_2Cl_2$, the two-dimensional charge distribution in (100) plane is plotted in figure 4. Furthermore, by consideration of the electronic charge distribution plot, one can claim the covalence origin for the Hg-S bonds and ionic nature for the Hg-Cl bonds. The presence of covalent bonding between mercury and strongly polarized sulphur atoms leads to the high magnitude of refractive index in the title compound.[3] The covalent bond arises due to the significant degree of the hybridization and the electro-negativity differences between atoms.

Conclusions

The band structure of gyrotropic α-$Hg_3S_2Cl_2$ is investigated by means of the first-principles calculations in the framework of the DFT. Using local density approximation within Hubbard correction, the atomic positions are relaxed so as minimize the forces acting on each atom. The analysis of DOS/PDOS indicates the origin of electronic properties in α-$Hg_3S_2Cl_2$ that connected with rare space symmetry group of mentioned crystal. The calculated electronic charge density distribution confirms the covalence and ionic nature for the Hg-S and Hg-Cl bonds respectively. The title compound combine important features needed for good X-ray and γ-ray detection applications, namely high atomic numbers and specific densities, wide band gap of 3.198 eV. It is in good agreement with experimental results. This may open a new area in applications of corderoite family compounds as multifunctional materials.

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