Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2009, 1 (2)1-11
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X

Kinetic spectrophotometric method for the determination of tetracycline hydrochloride in pharmaceutical formulations

Nagwa H. S. Ahmida[a*], Ebtisam El-Hasheme[a], N. El-Enany [b]and F. Belal[b]

[a] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Al-Arab Medical University, Benghazi-Libya.*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt*

Abstract

A simple and senstive kinetic spectrophotometric method was developed for the determination of tetracycline in capsule dosage forms. The method is based on the reaction of tetracycline with potassium permanganate in alkaline medium to form a green color of potassium mangante at room temperature. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 610 nm. The absorbance-concentration plot was rectilinear over the range of 1.0-30.0 µg/ml with limit of detection (LOD) of 0.96 µg/ml and limit of quantification (LOQ) 3.22 µg/ml. Different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The determination of tetracycline by the fixed-concentration and rate-constant methods is also feasible with the calibration equations obtained, but the fixed time method has been found to be more advantageous. The proposed method was further applied to the determination of the drug in formulations. The results obtained were in good agreement with those obtained using the official method. A proposal of the reaction pathway was postulated.

Key Words: Tetracycline, Kinetic spectrophotometry, potassium permanganate, dosage forms.

Introduction

Tetracycline, fig 1, is chemically 4-(dimethyl amino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octa hydro-3, 6, 10, 12, 12a-penta hydroxy-6-methyl-1, 11-dioxo-2-naphthacene-carboxamide [**1]**. Tetracycline is broad spectrum antibiotic. It inhibits cell growth by inhibiting translation. It binds to **16 S** part of the **30 S** ribosomal subunit & prevents the amino-acyl t-RNA from binding to the site of the ribosome [1, 2].

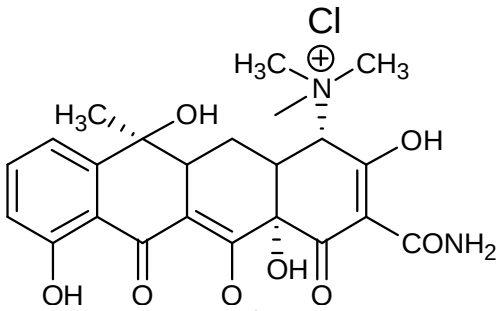


Fig 1: **Chemical structure of tetracycline hydrochloride**

Several methods have been reported for the determination of tetracycline in dosage forms including: Microbiological assay [*3*], non- Aqueous volumetric titration [4], TLC densitometry with fluoresence [5]. Also High Performance Liquid Chromatography (HPLC) methods have been used with different detections. Such as UV- detection [6-10] or fluorescence detections using post column derivatization. [11-15], tandem mass spectrometry [16-21] chemiluminescence detection [22-23] and electrochemical detection [24-25].

Capillary electrophoresis methods with UV-detection, at (360 nm), was utilized for simultaneous determination of tetracycline in milk, serum, urine [26], honey [27] and in fish samples [28]. A differential pulse polarographic method was also used for the determination of tetracycline in presence of its degradation products [29]. Numerous flow injection method with amperometric [30] and chemiluminometric [31] detections are also used for tetracycline determination in pharmaceutical preparation and honey.

In addition, various colorimetric methods were reported for determining of tetracycline. These methods included coupling (and complexing) of tetracycline with different reagents such as: urany acetate [*32*], 2,2-diphenyl-1-picrylhydrazol [33] and sodium cobalite nitrite [34] copper ion [35], magnesium ion [36], aluminium ion [37] and vanadium ion [38]. Some of colorimetric methods are rather insensitive and subject to interference from other materials. Therefore, Vetuschi C. and Rango G., 1990 [39], described derivative ultraviolet spectrophotometric assay for simultaneous determination of tetracycline and their anhydro-derivatives, in pharmaceutical preparations.

The literature is still poor in analytical procedures based on kinetics, especially for pharmaceuticals or biological fluids. However, some specific advantages in the application of kinetic methods can be expected such as, selectivity due to the measurement of the evolution of the absorbance with the time of the reaction instead of the measurement of a concrete absorbance value [40]. Potassium permanganate has been frequently utilized in the field of pharmaceutical analysis. Many pharmaceutical compounds have been determined kinetically through this approach such as oxamniquine [41], salbutamol [42] and metronidazol [43]. The aim of the present work was to investigate the reaction between tetracycline and alkaline $KMnO_4$ kinetically in an attempt to evaluate the drug content in capsule dosage forms. The results obtained were promising.

Materials and Methods

Apparatus

A Jena Model UV-visible Spectrophotometer (Jena, Germany) was used to measure the absorbance at wavelength 610 nm, using 1cm quartz cells.

All chemicals and reagents were of analytical grade.
• Tetracycline pure sample was kindly provided by Prof. Belal, Mounasoura University.
• Tetracycline hydrochloride capsules containing 250 mg: Julphar (UAK), AL-Naser (Egypt) and Remedica (Cyprus) were obtained from commercial sources in the local market.
• Potassium permanganate (Merck, Darmstadt, Germany): 0.01 mol L^{-1} aqueous solution was freshly prepared.
• Sodium hydroxide (BDH, Poole, England): 0.5 mol L^{-1} aqueous solution.
• A stock tetracycline solution was prepared by dissolving 10.0 mg of pure drug in 100 mL of distilled water.

Recommended procedure

Transfer aliquot volumes of tetracycline standard solution covering the working concentration range from 1.0 to 30.0 µg mL^{-1} into 25 mL volumetric flasks; add 3.0 mL of 0.01 mol L^{-1} potassium permanganate followed by 3.5 mL of 0.5 mol L^{-1} NaOH and shake well, then make up to the mark with water. Allow the reaction mixture to stand for 20 min. Measure the absorbance of the resulting solution at 610 nm against a reagent blank prepared simultaneously. Plot the values of the absorbance against the final concentration in µg mL^{-1} to get the calibration curve. Alternatively, derive the corresponding regression equation.

Procedure for Tetracycline capsules

Empty the contents of 10 capsules and mix well. Transfer a weighed quantity of the powdered capsules equivalent to 10 mg of tetracycline into 100 mL volumetric flask and made up to the mark with water. The content of the flask was stirred magnetically for 10 minutes, then proceed as described under "Recommended Procedure".

Results and Discussion

The absorption spectrum of $KMnO_4$ in basic medium shows an absorption bands at 510, 530 and 550 nm (fig 2). The addition of aqueous solution of tetracycline to $KMnO_4$ solution in basic medium causes a change in the absorption spectrum of $KMnO_4$ (producing of green color) with a new characteristic bands at 610 nm.

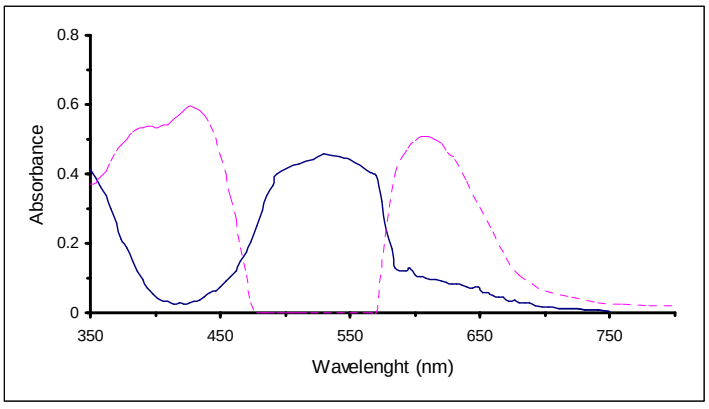


Fig 2: Absorption spectra of (—) 5.0×10^{-3} M $KMnO_4$ solution in alkaline medium and (…) of tetracycline-$KMnO_4$ product

Optimization of Variables

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully

studied and optimized. Such factors were changed individually while the others were kept constant. These factors include concentration of the reagents ($KMnO_4$ and NaOH) and time of reaction.

Effect of $KMnO_4$ Concentration

It was found that, increasing the volume of $KMnO_4$ solution (0.01 M) resulted in a gradual increase in the absorbance value up to 3 ml, after which a constant slight decrease in the absorbance value was observed. Therefore, 3 ml of 0.01 M $KMnO_4$ was chosen as the optimal volume of the reagent (Fig. 3)

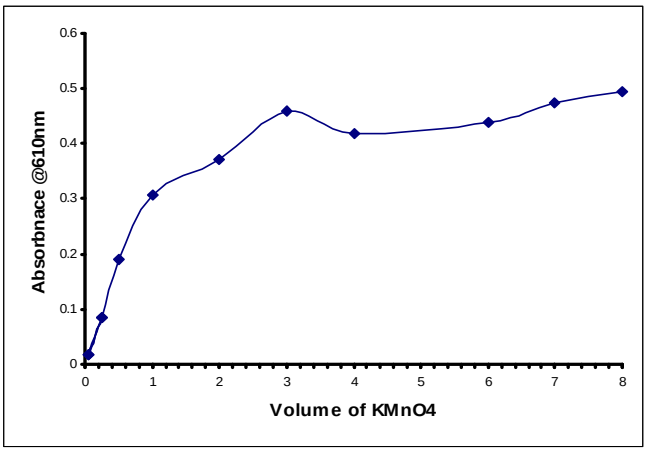


Fig 3: Effect of volume of (0.01 M) $KMnO_4$ on the absorbance intensity of 2.08×10^{-5} M tetracycline and 0.02 M NaOH measured at room temperature

Effect of NaOH Concentration

The influence of NaOH volume on the absorbance of the reaction product was also studied. It was found that increasing the volume of 0.5 M NaOH resulted in a corresponding increase in the absorbance of the reaction product up to 3 ml. Further increase of the alkali volume above 5 mL resulted in a slight decrease of the absorbance. Thus, 3.5±0.5 ml of 0.5 M NaOH was established as the most suitable volume for this study (Fig. 4).

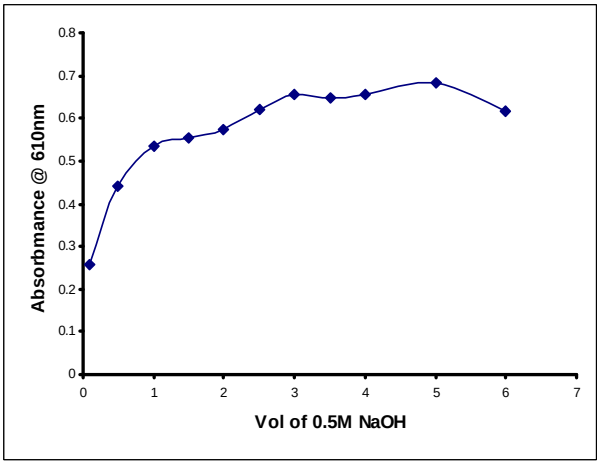


Fig 4: Effect of sodium hydroxide concentration on the reaction product of 2.08×10^{-5} M tetracycline and 1.2×10^{-3} M $KMnO_4$ measured at room temperature at 610 nm

Kinetic of the reaction

Because the intensity of the color increased with time (fig 5), it was used as the basis for a useful kinetic method for the determination of tetracycline. Under the optimized conditions of $KMnO_4$ and NaOH basis of experimental observations, kinetics equation for reaction may be written as:

$$\textbf{Rate} = K\,[\text{Drug}]^n\,[KMnO_4]^m$$

The concentration of $KMnO_4$ was 1.2×10^{-3} M, the above equation will reduce to

$$\textbf{Rate} = K`\,[\text{Drug}]^n$$

where **K`** is the pseudo-rate constant, **n** is the order of reaction. The logarithmic form of the equation may be written as:

$$\textbf{Log rate} = \log K` + n \log [\text{Drug}]$$

The initial rates of reaction were determined at different concentrations of tetracycline by measure the slops of the initial tangent of the absorbance at 610 nm- time curves during the first 30 min (fig 5). The results are summarized in table (1) [44].

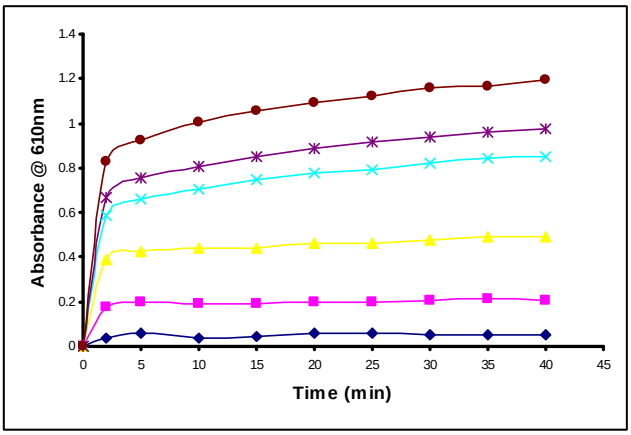


Fig 5: Absorbance- time curves for the initial rate of the reaction at varying concentration of tetracycline (♦) 2.08×10^{-6} M, (■) 8.32×10^{-6}M, (▲) 2.08×10^{-5}M, (×) 4.16×10^{-5}M, (ж) 4.99×10^{-5} M and (●) 6.24×10^{-5}M, keeping [$KMnO_4$]= 1.2×10^{-3}M and [NaOH]=0.07M

Table 1: Initial rate of reaction at different concentrations of tetracycline keeping [$KMnO_4$] and [NaOH] constants:

Log [Drug]	Log Rate
-6.080	-5.155
-5.682	-5.398
-5.080	-5.00
-4.779	-4.523
-4.682	-4.398
-4.478	-4.046
-4.381	-3.971
-4.302	-3.914
-4.235	-3.864
-4.205	-3.840

The plot of **log rate** versus **log [Drug]** gave the following linear equation:

Log rate = 1.1363 log [Drug] + 2.7399

With coefficient of correlation r^2 = 0.9949. The value of **n** in equation indicated that the reaction is first order with respect to tetracycline and the rate constant (K`) is 549.4 s^{-1}.

Evaluation of Kinetic Methods

Several experimental were run to obtain tetracycline concentration, using rate data, rate constant, fixed- absorbance and fixed-time methods [**45,46**], and the most suitable analytical method was selected taking into account the applicability and sensitivity (slop of the calibration graph, the correlation coefficient and the intercept).

1. Rate Constant Method

The best way to obtain an average K` value for the reaction, is to plot the logarithm of the concentration versus time. A graph of **Log (A)** versus **time** for tetracycline in the concentration range 1.0 -30.0 µg/mL ($2.08\times 10^{-6}$ – $6.24\times10^{-5}$ M) (fig 5), obtained pseudo first rate constant **K`** corresponding to different tetracycline concentrations. These **K`** values were calculated from the slopes of curves multiplied by -2.303 (table 2).

Table 2: Values of K` calculated from slops of Log A versus t graphs at 610 nm

[Drug] (M)	Equation	K` /s^{-1}
2.079×10^{-6}	Log A = 4×10^{-5} t -1.359	-9.212×10^{-5}
8.318×10^{-6}	Log A = 3×10^{-5} t – 0.7389	-6.909×10^{-5}
1.664×10^{-6}	Log A = 4×10^{-5} t – 0.4803	-9.212×10^{-5}
2.079×10^{-6}	Log A = 4×10^{-5} t – 0.3902	-9.212×10^{-5}
3.327×10^{-6}	Log A = 6×10^{-5} t – 0.2777	-1.3818×10^{-5}
4.159×10^{-6}	Log A = 6×10^{-5} t – 0.2035	-1.3818×10^{-5}
4.991×10^{-5}	Log A = 6×10^{-5} t – 0.1441	-1.3818×10^{-5}
5.822×10^{-5}	Log A = 7×10^{-5} t – 0.1064	-1.6121×10^{-5}
6.238×10^{-5}	Log A = 6×10^{-5} t – 0.0518	-1.3818×10^{-5}

Regression of **[tetracycline]** versus **K`** gave the following equation:

$$\mathbf{K` = -1.8544\ [Drug] - 4\times10^{-5}} \qquad \mathbf{r^2 = 0.278}$$

2. Fixed Absorbance Method

Reaction rate data were recorded for different tetracycline concentration in the range 1.0-30.0 µg/mL. A preselected of the absorbance (0.85) was fixed, and the time was measured in seconds (Table 3).

The reciprocal of time (1/t) versus the initial concentration of tetracycline was plotted and the following equation of calibration was obtained:

$$\mathbf{1/t = -5.3\times10^{-3} + 132.49\ [Drug]} \qquad \mathbf{r^2 = 0.9318}$$

The range of tetracycline concentration that giving the most satisfactory results was limited, therefore this method was abandoned.

Table 3: Values of reciprocal of time (1/t) taken at fixed absorbance value (0.85) for different concentrations of tetracycline at 610 nm

t (s)	1/t	[Drug]
2400	4.17×10^{-4}	4.16×10^{-5}
900	1.11×10^{-3}	4.99×10^{-5}
480	2.083×10^{-3}	5.82×10^{-5}
300	3.33×10^{-3}	6.24×10^{-5}

3. Fixed Time Method

The absorbance of the green colored solution at 610 nm was recorded for different concentrations of tetracycline ranging from 1.0-30.0 µg/mL. The calibration graphs of absorbance versus initial concentrations were established at fixed time, 30 min with a regular interval of 5 min. The regression equations assembled in table 4.

Table 4: Regression equations for tetracycline at fixed time

Time (s)	Calibration equation	Correlation Coefficient (r^2)
120	Y= 11867 X + 0.0832	0.9768
300	Y = 13204 X + 0.091	0.9834
600	Y = 14779 X + 0.0749	0.9859
900	Y = 15713 X + 0.0715	0.9889
1200	Y = 16234 X + 0.0781	0.9904
1500	Y = 16874 X + 0.0718	0.9919
1800	Y = 17390 + 0.731	0.9912
2100	Y = 17675 X + 0.0769	0.9910

It is evident from table 4, that the most acceptable linearity was obtained when the calibration graphs were plot at 20 min (there is a negligible difference between slop-started at 20 min to 30 min). The fixed time method recommended for the tetracycline analysis. The calibration graph was linear in the range of 1.0-30.0 µg/mL. Analysis of data gave the following equation

$$\mathbf{A = 0.0338\ C + 0.0781} \qquad \mathbf{r^2 = 0.9904}$$

where A is the absorbance and C is the concentration of the drug in µg/mL. Validation of the proposed method was evaluated by statistical evaluation of the data. It was found that the standard deviation of the residual ($S_{y/x}$) is 3.75×10^{-2}, the standard devation of the intercept (S_a) is 2.33×10^{-2} and standard deviation of the slop (S_b) is 1.26×10^{-3}. The small values of the figures point out to low scattering of the point around the calibration curve and high precision of the proposed method.

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be detected. The LOD was found to be 0.96 µg/mL (1.996×10^{-6} M), according to the 3s/m definition [47], where (s) is the standard deviation (n=6) of the signal from 16.0 µg/mL tetracycline aliquots. The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision and was found to be 3.22 µg/mL (6.69×10^{-6} M). The accuracy and precision of the method were evaluated by analyzing standard solution of tetracycline. The results for the

method were also applied to tetracycline capsules Table (5). The official method [4] is based on non-aqueous titration of the drug using perchloric acid as a titrant and brilliant green as indicator.

Table 5: Application of the proposed and official methods to the determination of tetracycline in pure and dosage forms:

Preparation	Taken (µg)	Found [(a)] (µg)	Recovery (%)	Official Method[(b)]
Tetracycline HCl	4	3.95	98.67	
	8	7.96	99.50	
	12	12.09	100.78	
	16	16.29	101.81	
	24	24.14	100.60	
Mean ± s.d			100.07 ± 0.95	99.58 ± 1.20
t-test			0.64	
F-test			0.6190	
Tetracycline HCl capsule (Julphar UAE)	8	8.29	103.63	
	12	12.32	102.64	
	16	16.23	101.42	
	24	24.40	101.65	
Mean ± s.d			101.90 ± 1.01	103.34 ± 1.00
t-test			1.87	
F-test			0.94	
Tetracycline HCl capsule (Remidica, Capurs)	8	7.94	99.21	
	12	11.95	99.56	
	16	16.15	100.92	
	24	24.05	100.21	
Mean ± s.d			100.23 ± 0.75	99.43 ± 0.92
t-test			1.27	
F-test			0.71	
Tetracycline HCl capsule (Al-Naser, Egypt)	8	8.13	101.58	
	12	12.25	102.11	
	16	16.29	101.81	
	24	24.33	101.36	
Mean ± s.d			101.72 ± 0.32	101.48 ± 0.88
t-test			0.52	
F-test			0.14	

[a]. each value is the average of five separated determinations.
[b] each value is the average of three separated determinations.
- The tabulated values of t and F values are 1.943 and 6.94 respectively at p= 0.05 [**48**].

Proposal of the reaction pathway

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [49]. The absorbance of the reaction product was alternatively measured in the presence of excess of either $KMnO_4$ or tetracycline. A plot of log absorbance (**log A**) versus each of **log**

[$KMnO_4$] and **log [tetreacycline]** gave straight lines, the values of the slops are 0.9375 and 0.8629 respectively (fig 6). Hence, it concluded that, the molar reactivity of the reaction is 0.86: 0.9, i.e. the reaction proceeds in the ration of 1:1.

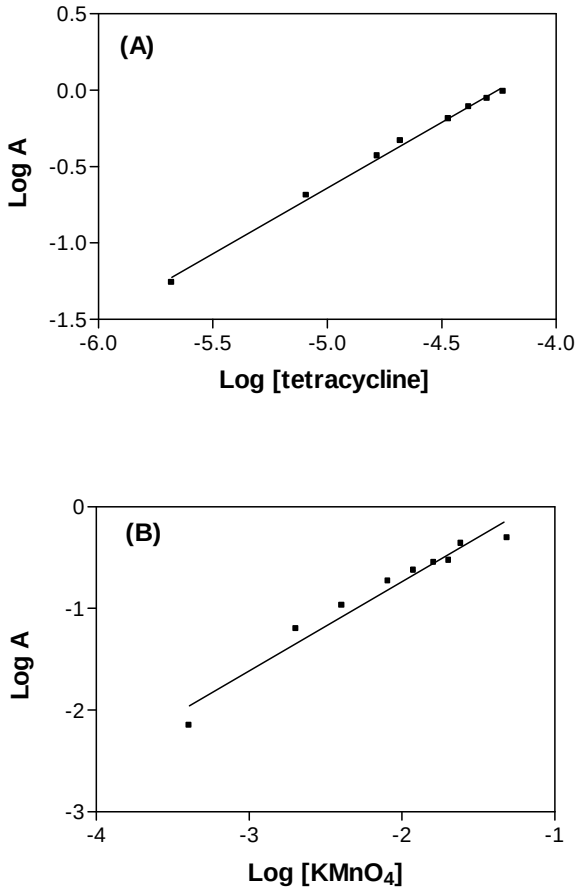
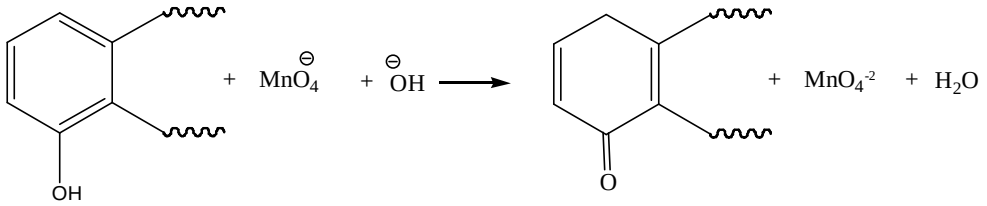


Fig 6: Limiting logarithmic plots for the molar ratio. (A) log A vs. log [$KMnO_4$] with [tetracycline] kept at M 1.66×10^{-5}M. (B) Log A vs. log [tetracycline] with [$KMnO_4$] kept at 1.2×10^{-3}M.

Based on the obtained molar reactivity and by analogy to similar reports [50] the phenolic group of tetracycline is oxidisd by the $KMnO_4$/ NaOH system into the corresponding quinonoid structure where by the permanganate ion is reduced to the manganate ion which is coloured species. The reaction pathway is proposed to proceed as follows:



Scheme 1: **The proposed mechanism of reaction between tetracycline and potassium permanganate in alkaline medium**

Conclusion

The concentration of tetracycline was studied kinetically using different approaches: the reaction rate method, rate constant and fixed time methods. In the fixed time method, the 20 min time interval proved to be the most suitable, which was confirmed by satisfactory values of the correlation coefficients (r^2) and slopes of the obtained calibration plots. Moreover, the proposed method is simple and sensitive, and could be adopted to the analysis of dosage forms. It allows the determination of as low as 3.22 µg/ml with good accuracy.

References

[1] Wilson and Gisvold's Textbook **"**Organic Medicinal and Pharmaceutical chemistry" edited by John H. Block and John M. Beale, Wolters Kluwer, London , UK, **2004**, pp**.** 341 -346.
[2] Martindale "The Extra Pharmacopeia -The Complete Drug Reference" edited by Sean C sweetman , **35**th ed. ,Pharmaceutical press, London, UK, **2007**, pp. 266-268.
[3] British Pharmacopoeia , Her Majesty's Stationary Office, The Pharmaceutical Press, London, UK, **1998**, vol II ,Appendix A103.
[4] The international pharmacopoeia (Quality Specifications) volume 2, 3rd ed.; World Health Organization ,Geneva**, 1981,** pp. 266-270**.**
[5] W. Naidong; S. Hua; E. Rocts; J. Hoogmartens *J. Pharm Biomed Anal***., 2003**, 33,1, 85.
[6] A. R. Long; L. C. Hsieh; M. S. Malbrough; C. R. Short; Barker S. A *J. Assoc Off Anal. Chem*., **1990,** 73, 3, 379.
[7] J. O. De Beer; J. Hoogmartens *J. Pharm. Biomed Anal.*, **1993**, 11,11-12, 1239.
[8] L. Wang; X. S Zhang; Z. X. Xu; X. G. Shao *Se Pu*., **2002**, 20, 1, 49. {article in Chinese}.
[9] A. L. Cinquina.; F. Longo; G. Anastassi; L. Giannetti; Cozzani R. *J. Chromatogr A*., **2003**, 987 (1-2) 227.
[10] J. Li; L. Chen; X. Wang; H. Jin; L. Ding; K. Zhang; H. Zhang *Talanta*, **2008**, 75, 5, 1245.
[11] S. Croubels; C. Van Peteghem; W. Baeyens *Analyst*, **1994**, 119, 12, 2713.
[12] A. Pena; A. Carmona; A. Barbosa; C. Lino; I. Silveira; B. Castillo *Pharm Biomed Anal*., **1998**, 18, 4-5, 839.
[13] A. L. Pena; C. M. Lino; M. I. Silveira *J. AOAC Int*., **2003**, 86, 5, 925.
[14] S B. F. Spisso; E. Oliverira; A. L. Jesus; M. A. Jr. De Araujo; M. A. Monteiro *Anal. Chim Acta.*, **2007**, 581, 1, 108.
[15] K. Fujita; H. Ito; M. Ishihara; S. Inukai; H. Tanaka; M. Taniguchi *Shokuhin Eiseigaku Zasshi.*, **2008**, 49, 3, 196.
[16] W. H. Farrington; J. Tarbin; J. Bygrave; G. Shearer *Food Addit Contam*., **1991**, 8, 1, 55.
[17] P. J. Kijak; M. G. Leadbetter; M. H. Thomas; E. A. Thompson *Biol. Mass Spectrom*, **1991**, 20, 12, 789.
[18] R. Ishii; M. Horie; M. Murayama; T. Maitani *Shokuhin Eiseigaku Zasshi*., **2006**, 47, 6, 277.
[19] H. De Ruyck; H. De Ridder *Rapid Commun Mass Spetrom*, **2007**, 21, 9, 1511.
[20] B. Shao; X. Jia; Y. Wu; J. Hu; X. Tu; J. Zhang *Rapid Commun. Mass Spectrom*, **2007**, 21, 21, 3487.
[21] X. Jia; B. Shao; Y. Wu; Y. Yang; J. Zhang *J. AOAC Int.*, **2008**, 91, 2, 461.
[22] G. H. Wang; H. Cui; H. S. Zheng; J. Zhou; L. J. Liu; X. F. Yu *J. Chromatogr B Analyt. Technol Biomed Life Sci.*, **2005**, 824, 1-2, 57.

[23] V. R. Santiago; P. I. Sanchez; F. Franceschelli; G. M. Martinez; M. D. Gil Garcia *J. Chromatogrr A*., **2007**, 1167, 1, 85.
[24] F. Zhao; X. Zhang; Gan Y. *J. Chromatogr A.*, **2004**, 1055, 1-2, 109.
[25] T. Charoenraks; S. Chuanuwatanakul; K. Honda; Y. Yamaguchi; O. Chailapakul *Anal. Sci.*, **2005**, 21, 3, 241.
[26] C. L. Chen; X. Gu *J. AOAC Int*., **1995**, 87, 6, 1369.
[27] S. Casado-Terrones; A. Segura-Carretero; S. Busi; G. Dinelli; A. Fernandez-Gutierrez *Electrophoresis*, **2007**, 28, 16, 2882.
[28] P. Kowalski *J. Pharm Biomed Anal.*, **2008**, 47, 3, 487.
[29] S. Sabharwal; K. Kishore; P. N. Moorthy *J. Pharm Sci.*, **1988**, 77, 1, 78.
[30] S. Treetepvijit; S. Chuanuwatanakul; Y. R. Einaga Sato; O. Chailapakult *Anal Sci.*, **2005**, 21, 5, 531.
[31] S. A. Halvatzis; M. M. Timotheou-Potamia; A. C. Calokerinos *Analyst*, **1993**, 118, 3, 633.
[32] U. Saha; A. K. Sen; T. K. Das; S. K. Bhowal *Talanta*, **1990**, 37, 12, 193.
[33] K. M. Emara; H. F. Askal; Saleh G. A. *Talanta*, **1991,** 38, 11, 1219.
[34] M. S. Mahrous; M. M. Abdel-Khalek *Talanta*, **1984**, 31, 4, 289.
[35] U. Saha *J. Assoc. Off Anal Chem.*, **1987**, 70, 4, 686.
[36] W. B. Chang; Y. B. Zhao; Y. X Ci; L. Y. Hu *Analyst*, **1992**, 117, 8, 1377.
[37] S. Liawraangrath; B. Liawraangrath; S. Watanesk; W. Ruengsitagoon *Anal. Sci. J*., **2006**, 22, 15.
[38] M. M. Abdel-Khalek; M. S. Mahrous *Talanta*, **1983**, 30, 10, 792.
[39] C. Vetuschi; G. Ragno *Farmaco* ,**1990**, 45, 6, 757.
[40] A. Espinosa- Mansilla; M.I. Acedova- lenzuela; F. Salinas; F. Canada *Anal. Chim. Acta*, **1998**, 376**,** 365.
[41] M. Rizk; F. Belal; F. Ibrahim; S.M. Ahmed; N.M. El-Enany *J. Pharm. Biomed. Anal.*, **2000**, 23, 503.
[42] N. El-Enany; F. Belal; M. Rizk *Chem. Anal. (Warsaw)*, **2004**, 49, 587.
[43] N. H. S. Ahmida; B. B. Elbarasi Negia; N. El-Enany; F Belal *MJPS*, **2008**, 24, 1, 34.
[44] A. Weisberger; S. Friess; E.-S. Lewis Techniques of Organic Chemistry, vol. III, Interscience, New York, USA, **1953**.
[45] K.B. Yatsimirskii "Kinetic Methods of Analysis", Pergamon Press, Oxford, UK, **1966**.
[46] H. A. Laitimen; W. E. Harris "Chemical Analysis", 2nd ed., Mc-Graw- Hill, New York,.USA, **1975.**
[47] Hasebe K. Osteryoung *J. Anal. Chem*., **1975**, 47, 2412.
[48] J. C. Miller; J.-N. Miller Statistics for Analytical Chemistry, 5th ed., Wiley, New York, USA, **2005,** pp. 256.
[49] J. Rose Advanced Physico-Chemical Experiments, Pitman, London., UK, **1964**, pp. 67.
[50] N. El-Enany; F. Belal; M. Rizk *Il Farmaco*, **2002,** 57, 641.