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Spectrophotometric investigation of the formed chelate between timonacic and palladium(**II**) and its analytical applications

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Abstract

A simple spectrophotometric method for the determination of timonacic is presented. The procedure is based on the chelate formation with palladium(**II**) chloride in buffered medium. The optimum conditions for the complex formation were ascertained and the method was developed for the determination of timonacic in the concentration range of 28–48 μg ml^{-1}. The emperical formula of the formed complex was determined, by applying different spectrophotometric methods, at optimum pH of 4.8 and an ionic strength of $\mu = 0.5$. The stoichiometric ratio was found to be 2:1 (timonacic/palladium) as calculated by the mole ratio, continuous variations and Asmus methods. The continuous variations and Nash methods were applied for the determination of the conditional stability constant of the formed yellow-water soluble complex and was found to be 3.27×10^7. The proposed methods was found to be suitable for the determination of timonacic in bulk and in its pharmaceutical tablets.

Keywords: Chelation; Conditional stability constant; Spectrophotometric; Stoichiometric ratio; Timonacic

1. Introduction

Timonacic (TC) (thiazolidine-4-carboxylic acid) is used as an adjuvant in the treatment of acute and hepatic disorders. It has also been used for the treatment of some cases of cancer, through the induction of the reverse transformation [1].

Several electrochemical methods have been reported for the determination of (TC) including polarographic titration [2] and various voltametric techniques [3,4]. The D- or L- forms of (TC) were separated by reverse phase HPLC method [5]. Recently, (TC) was determined spectrophotometrically through the formation of coloured dialkylvinyl amino quinones [6] or through an ion pair formation with quinones [7].

There are no published reports on the use of metal ions to quantitate (TC). The present report based on the chelate forming ability of (TC) with palladium(**II**) chloride (Pd). The optimum conditions, for the chelate formation (pH, temperature etc.), were established. At the same time, it seemed worthwhile to examine the TC-Pd complex in more detail to assure the utility of the reaction for the spectrophotometric determination of (TC) in bulk and pharmaceutical tablets.

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2. Experimental

2.1. Instrumentation

The spectrophotometric measurements were carried out using Perkin-Elmer double beam UV-VIS spectrophotometer Model 550S, attached to a Hitachi recorder Model 561, with a fixed slit width of 2 nm and using 1 cm quartz cells. The pH measurements were carried out using Schott-Gerate pH meter Model CG 710, checked for pH setting using standard solutions of pH 4 and 7.

2.2. Materials and reagents

Analytical reagent grade of hydrochloric acid, sodium acetate, acetic acid, potassium chloride and double-distilled water were used. Palladium chloride solution (5×10^{-3} M) was prepared by weighing about 884 mg palladium(**II**) chloride (sigma Chem. Co., Milwaukee, WI-USA) in a 200 ml beaker, adding 0.8 ml concentrated hydrochloric acid followed by stepwise addition of 50 ml of hot water. Heating and stirring were continued till the complete dissolution. The solution was then cooled and diluted to a 100 ml with distilled water, in a 100 ml volumetric flask. Walpole's acetate buffer solutions [8], pH range 3.6–5.6, were prepared by mixing different volumes of 0.2 M acetic acid with 0.2 M sodium acetate solutions. The pH of the solutions was adjusted by using pH meter. Timonacic was synthesized according to the procedure of Ratener et al. [9] and checked against standard material supplied as a free gift from South Egypt Drug Industries Co. (SEDICO-6 October City, Egypt). Timonacic standard solution (0.4 mg ml^{-1}) was prepared by dissolving 40 mg (TC) in 100 ml hot distilled water. The ionic strength (μ) of the final solutions used for the spectrophotometric measurements was kept constant at 0.5 M by the addition of 2.5 M potassium chloride solution.

Commercial tablets of (1) Hepatone (Sedico Pharm. Co., BN. 392109) and (2) Hepargen (Medical Union Pharmaceuticals (Ismailia-Egypt) under licence by Syntex Pharm. AG, Switzerland, BN. 924901), were labelled to contain 100 mg of timonacic. The tablets were purchased from the local market.

2.3. General procedure and construction of calibration graph

To a set of 10 ml volumetric flasks, different volumes of (TC) standard solution (0.7–1.2 ml, in 0.1 ml increment) were quantitatively transferred. To each flask, 1 ml buffer solution of pH 4

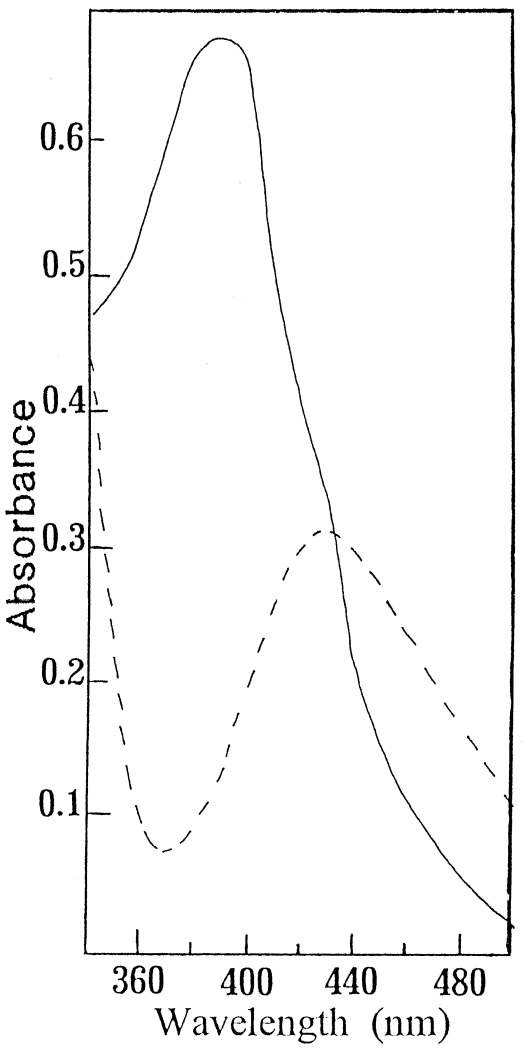


Fig. 1. Absorption spectra of palladium(**II**) chloride (1.5×10^{-3} M) (- - -) and the complex formed through the reaction of 44 μg ml^{-1} timonacic with palladium(**II**) chloride (——).

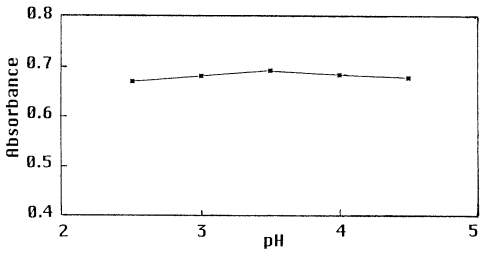


Fig. 2. Effect of pH on the absorbance of [TC-Pd(**II**)] complex, [(TC) $= 40$ μg ml^{-1}].

followed by 3 ml $PdCl_2$ solution and 2 ml potassium chloride (2.5 M) solution were added. The reaction mixtures were left to stand at room temperature for 5 min. The flasks were then completed to volume with water. The absorbance of the resulting solutions was then measured at 385 nm against reagent blank simultaneously prepared.

2.4. Procedure for the assay of commercial tablets

Twenty tablets were weighed and powdered. A quantity of the powder equivalent to 40 mg of (TC) was weighed and transferred to a 100 ml volumetric flask with the aid of about 50 ml of hot water. The flask was sonicated for 15 min in an ultrasonic water bath. The flask was completed to volume with water and filtered. Aliquots from the filtrate were used for the application of the proposed general procedure.

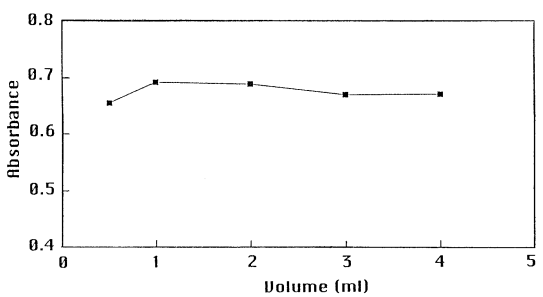


Fig. 3. Effect of buffer volume on the absorbance of [TC-Pd(**II**)] complex, [(TC) $= 40$ μg ml^{-1}].

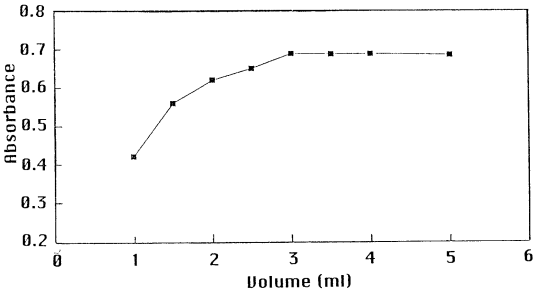


Fig. 4. Effect of Pd(**II**) concentration on the absorbance of [TC-Pd(**II**)] complex, [(TC) $= 40$ μg ml^{-1}].

3. Results and discussion

Transition elements were found to form stable complexes with many ligands containing heteroatoms. There is preference for amines, halogens, CN^-, tertiary phosphrines and sulfides. Palladium(**II**), as one of the transition elements was found to form complexes of square or 5-coordinate shape with the general formula of ML_2X_2, where L is a neutral ligand and X a uninegative ion [10]. Palladium(**II**) was found to form stable complexes with many drugs; for example: phenothiazines [11,12], captopril [13] and *N*-acetyl-L-cysteine [14]. Theoretically and from what was mentioned above, (TC) could form chelate, through its sulphur atom, with palladium(**II**) chloride. Addition of palladium(**II**) chloride to (TC) produced yellow complex that is soluble in acetate buffer in the pH range of 3.6–5.6. The absorption spectrum was recorded over the range of 340–500 nm and showed a maximum absorbance at 385 nm (Fig. 1), which was therefore used for the

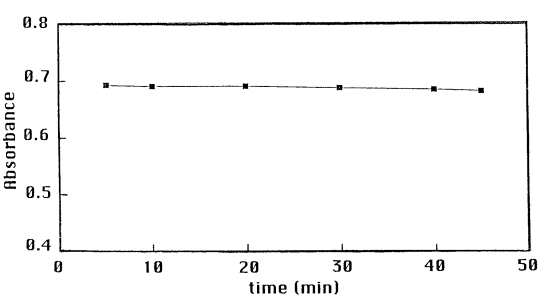
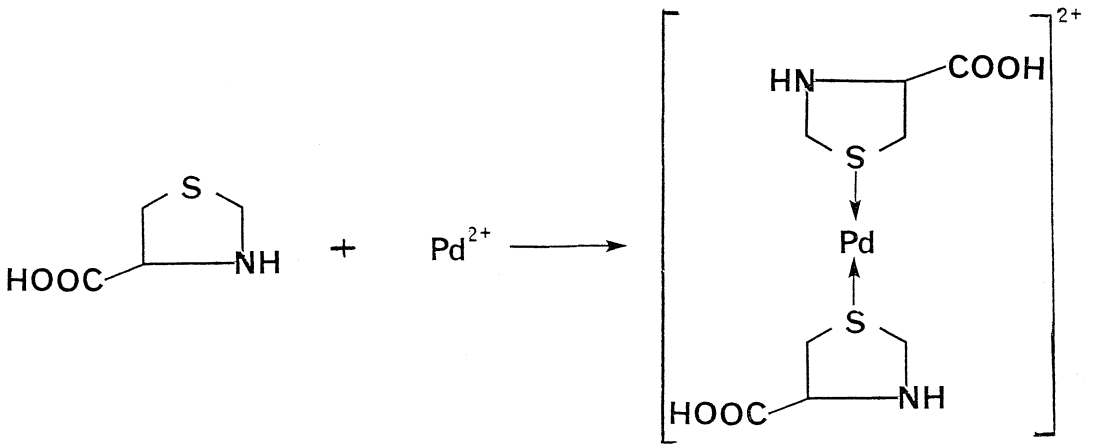


Fig. 5. Absorbance stability of [TC-Pd(**II**)] complex at room temperature, [(TC) $= 40$ μg ml^{-1}).



Scheme 1.

analytical determination. Timonacic is weakly UV-absorbing compound which does not absorb at 385 nm. At the same time, palladium(**II**) chloride has a low absorbance at the same wavelength (Fig. 1). Therefore, all measurements were performed against a reagent blank with correction for the cell blank as appropriate.

3.1. Study of the optimum reaction conditions

The reaction rate and the amount of TC-$PdCl_2$ complex produced were not greatly influenced by the change of the pH of the medium (Fig. 2). The shape of the absorption spectrum, the position of the absorption maximum and the apparent molar absorptivity of TC-PCl_2 complex do not vary with pH, which indicates that only one type of complex was formed. At the same time, using different buffer composition (Walpole's acetate or MaClivan's phosphate [8]) did not show any significant effect. In the present work, Walpole's acetate buffer was used to keep the pH constant throughout the study. The maximum sensitivity was obtained when a medium containing 1 ml of the acetate buffer of pH 4 was used (Fig. 3). Searching for better stability and more sensitivity, different surfactants were tried. The non-ionic and cationic surfactants such as methylcellulose, Triton X-100 and Brij had no effect, the anionic ones such as sodium lauryl sulfate decreased the sensitivity. Therefore, for a simple procedure there is no need to use surfactant.

An investigation on the effect of palladium(**II**) chloride concentration on the formation of TC-

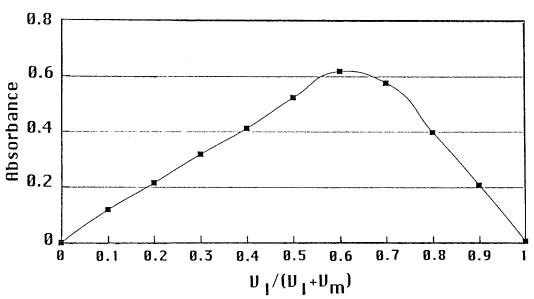


Fig. 6. Continuous variation plot for TC-Pd(**II**) (2×10^{-3}M) complex ratio (V_l, V_m, ligand and metal volume, respectively).

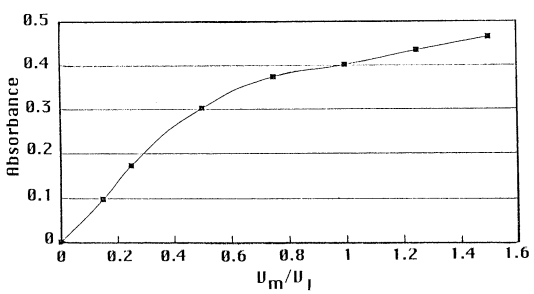


Fig. 7. Molar ratio plot for TC-Pd(**II**) (2×10^{-3} M) complex ratio (V_l, V_m, ligand and metal volume, respectively).

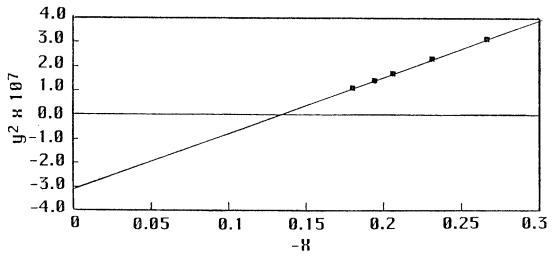


Fig. 8. Asmus method, [(TC) concentration = 24–48 μg ml^{-1}, Pd(**II**) = 1.5×10^{-3} M].

$PdCl_2$ complex showed that 3 ml of $PdCl_2$ solution was required to obtain maximum absorbance (Fig. 4). No effect was observed when the temperature was raised from room temperature to 60°C. Full color development was achieved immediately and its absorbance remained constant for at least 1 h (Fig. 5).

3.2. Composition of the complex

The reaction stoichiometry between (TC) and $PdCl_2$ has been determined spectrophotometrically by applying Job's method of continuous variations [15,16], mole-ratio method [16] and straight line method or Asmus method [17]. For Job's method, the plot reached a maximum value at a mole fraction of 0.66 which indicated the formation of a 2:1 (TC-Pd(**II**)) complex (Scheme 1 and Fig. 6). The plot obtained by the mole ratio method also confirm the formation of TC-Pd(**II**) complex in a mole ratio of 2:1, where a break point at 0.5 was obtained (Fig. 7). For more confirmation of the complex composition, Asmus method was applied. The Asmus method equation

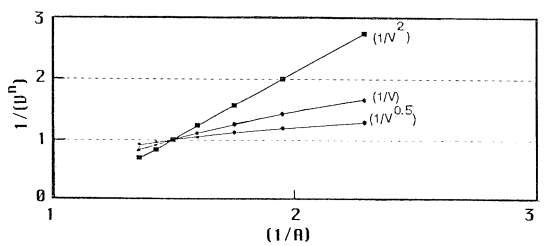


Fig. 9. Nash's method, [(TC) concentration = 24–40 μg ml^{-1}, Pd(**II**) = 1.5×10^{-3} M].

Table 1
Optical characteristics, Beer's law data and statistical analysis of TC/$PdCl_2$ complex

1.	λ max (nm)	385
2.	Beer's law limit (μg ml^{-1})	28.48
3.	Apparent molar absorptivity[a] (l mol^{-1} cm^{-1})	2.07×10^3
4.	Sandell's sensitivity (μg ml^{-1} per 0.001A)	2.58 x 10^{-2}
5.	Detection limit (μg ml^{-1})	0.595
6.	Regression equation	
(a)	Intercept	7.06×10^{-2}
(b)	Slope	1.55×10^{-2}
(c)	Correlation coefficient	0.9996
(d)	Variance S_σ^2	1.38×10^{-5}

[a] Calculated on the basis of the molecular weight of TC.

for the current reaction between (TC) and $PdCl_2$ can be presented as; $Pd^{2+} + n\,TC = PdTC_n$. By ploting $(1/V)^n$ versus $1/A$ (where V is the volume of (TC) and A is the absorption of the complex) a straight line was obtained only when $n = 2$ (Fig. 8), indicating that 2 moles of (TC) combine with one mole of $PdCl_2$ to form a 2:1 complex, confirming the previous results.

3.3. Conditional stability constant of the complex

By Job's method of equimolar solutions and from Fig. 6, the conditional stability constant (K') was calculated and found to be 3.27×10^7. The result was confirmed by using the graphical method of Nash [18], where, a straight line was resulted when plotting the square of the reciprocal of (TC) concentration against the reciprocal of (1 − absorbance ratio) (Fig. 9). From the graph, the equilibrium constant (K') was found to be 3.27×10^7, as calculated from the negative intercept on the ordinate. The values of log K' calculated from both the Job's and Nash methods were found to be identical as 7.51, indicating very good agreement between both methods and high stability of the complex.

3.4. Calibration graph and statistical analysis

By using the above spectrophotometric procedure, a linear regression equation was obtained. The regression plot showed a linear dependance

Table 2
Study of precision and accuracy of the proposed method

No.	Timonacic (μg ml^{-1})			S.D. (μg ml^{-1})	R.S.D. (%)	S.A.E. (μg ml^{-1})	95% confidence (μg ml^{-1})
	Added	Found	Recovery				
1	28	28.60					
		28.30					
		29.00					
		27.80					
		28.20					
Mean		28.38	101.36	0.45	1.58	0.20	0.56
2	32	32.50					
		31.90					
		31.70					
		32.80					
		31.40					
Mean		32.06	100.19	0.58	1.80	0.26	0.72
3	40	38.80					
		39.40					
		40.00					
		40.90					
		41.20					
Mean		40.04	100.10	1.00	2.51	0.45	1.25
Overall			100.55	0.68	2.00	0.30	0.84

S.D., standard deviation; R.S.D. %, relative standard deviation, S.A.E., standard analytical error.

of the absorbance over the Beer's law range given in Table 1. The molar absorptivity, slope, intercept, correlation coefficient, variance and detection limit obtained by the linear least square treatment of the results were listed in Table 1. The good linearity of the calibration graph and the

Table 3
Determination of timonacic in tablets

Commercial products	Label claim (μg ml^{-1})	Found (%)	Recovery[a](%)
Hepatone tablets	28	95.93	100.10
	30	95.89	100.00
	32	96.74	100.96
	36	97.03	99.80
	40	96.54	99.60
Mean		96.43	100.09
R.S.D. %		0.52	0.52
Hepargen tablets	28	100.28	100.70
	30	99.74	100.30
	32	101.14	99.75
	36	100.90	100.85
	40	100.65	100.05
Mean	100.54	100.33	
R.S.D. %	0.55	0.45	

[a] Standard addition of 50% to the nominal content.

negligible scatter of the experimental points were clearly evident from the values of the correlation coefficient and variance.

3.5. Accuracy and precision of the method

To test the accuracy and precision of the method, five successive measurements on the sample solution were carried out on three different (TC) concentrations. The small R.S.D. % and S.A.E. indicate high precision and good accuracy (Table 2).

3.6. Application to the analysis of commercial tablets

The proposed method has been applied for the analysis of (TC) in its commercial tablets. The recovery of the drug was tested by the standard addition method to the solution of the extracted tablets. The recovery was almost quantitative (Table 3). The proposed method was reproducible, simple and easily applied in the quality control laboratories.

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