J. Serb. Chem. Soc. 72 (1) 29–35 (2007)
JSCS–3531

UDC 547.581.9+543.4/.5:615.014.2
Original scientific paper

Spectrophotometric determination of thioctic (α-lipoic) acid in water and pharmaceutical preparations

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(Received 28 December 2005, revised 30 May 2006)

Abstract: A spectrophotometric method is described for the assay of thioctic acid. The method is based on the reaction between the drug and palladium(II) chloride. In this reaction, a yellow-coloured, water soluble product with a 1:1 stoichiometric ratio and an absorption maximum at 365 nm was formed. The stability of the formed complex depends on various factors (pH, reaction time, concentration of reagents, ionic strength). Based on these findings, a new method is suggested for the spectrophotometric determination of thioctic acid in pharmaceutical formulations. This method is simple, sensitive and reproducible.

Keywords: thioctic acid, spectrophotometric determination, injection solutions and tablets.

INTRODUCTION

Thioctic acid (α-lipoic acid), 1,2-dithiolane-3-pentanoic acid, is used extensively in the treatment of various diseases, such as alcoholic liver disease, mushroom poisoning, heavy metal poisoning, glaucoma, radiation injury and neurodegenerative disorders; it has been proposed that thioctic acid acts as an antioxidant and interferes with the pathogenesis of diabetic polyneuropathy.[1,2] Currently various methods, such as colorimetric assay,[3] gas chromatography,[4] GC-mass spectrometry,[5,6] high-performance liquid chromatography,[7–10] adsorptive stripping voltametry[11] and capillary electrophoresis[12] are reported for the determination of thioctic acid. The kinetic spectrophotometric method[13,14] was applied for determination of thioctic acid in pharmaceutical dosage forms (tablets) and human serum, based on the catalytic effect of this compound on the iodine–azide reaction.

The present paper reports a sensitive and reproducible spectrophotometric method for the determination of thioctic acid alone, in injection solutions and in

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doi: 10.2298/JSC0701029K

tablets. The method is based on the complexation reaction between palladium(II) chloride and thioctic acid in Britton–Robinson buffer. The optimum reaction conditions, spectral characteristics, stability constant and stoichiometry of the complex have been established. Since thioctic acid is officially used for the treatment of diabetic polyneuropathy, it seemed of interest to develop a spectrophotometric method for its determination in water and in pharmaceutical preparations in addition to the existing analytical methods.

EXPERIMENTAL

Apparatus

Absorption spectra and spectrophotometric measurements were carried out on a GBC, UV/VIS, Cintra 20 double-beam spectrophotometer (Australia), in 1 cm quarz cuvettes. A radiometer PHM 62 pH meter (Copenhagen), calibrated with appropriate standard buffer solutions, was utilized for pH measurements.

Reagents

All reagents were commercial products of the highest purity available: thioctic acid (MW 206.3), purity ≥ 99.3 % (Berlin-Chemie, Germany); palladium(II) chloride (MW 177.61, Merck); Berlithion^{R} 300 ED ampoules containing 300 mg thioctic acid/12 ml (Berlin-Chemie, Germany); Thioctacid^{R} 600 HR filmtablettes 600 mg thioctic acid/tbl (Homburg-Viatris, Germany).

Doubly distilled water was used throughout.

Solutions

Stock solutions of thioctic acid (5×10^{-3} M) were freshly prepared in water.

Palladium(II) chloride standard solution (1×10^{-2} M) was prepared by dissolving palladium(II) chloride in water (to which 0.60 ml of concentrated hydrochloric acid had been added) by warming the mixture on a water-bath. The solution was cooled at room temperature and diluted to volume with water in a 250 ml volumetric flask. This solution was standardized gravimetrically by precipitation with dimethylglyoxime.[15] The standardization of the palladium(II) chloride solution is not necessary when determining thioctic acid alone.

The ionic strength (μ) of the final solution for the determination was kept constant at 0.2 M, by the addition of a 2 M potassium chloride solution.

Britton–Robinson buffer solutions[16] covering the pH range from 2.0 to 10.0 were used.

For the analysis of thioctic acid in Berlithion^{R} 300 ED ampoules, 10 ml of one ampoule was diluted with water to 250 ml. The approximate concentration after dilution was 4.8×10^{-3} M.

For the analysis of thioctic acid in Thioctacid^{R} 600 HR filmtablettes, ten tablets were powdered.[13] A weighed amount of powder was dissolved in water; the samples were centrifuged for 5 min at 3000 rpm, the supernatant was separated and water was added to give a solution of approximate 5×10^{-3} M.

An aliquot of this solution was treated by the same procedure as described for the "calibration graph".

Procedure for the preparation of the calibration graph

A portion (0.1–1.0 ml) of stock solution (5×10^{-3} M) thioctic acid was placed in a 10 ml volumetric flask, 5 ml Britton–Robinson buffer solution pH 2.20, 1 ml potassium chloride, and 0.2 ml palladium(II) chloride were added, and the solution was diluted with water. The solution was mixed and after ten minutes the absorbance measured at 365 nm against a reagent blank. All measurements were performed at room temperature (25 ± 0.5 °C).

RESULTS AND DISCUSSION

Absorption spectra

The colored complex of thioctic acid (TA) and palladium(II) chloride was studied in Britton–Robinson buffer over the pH range 2.00–6.00. The absorption spectra of a solution containing the water soluble yellow complex of thioctic acid with palladium(II) were recorded over the wavelength range 300–500 nm. The complex gave a sharp absorption peak at 365 nm (Fig. 1, curve 3), where the absorbance of palladium(II) was low (curve 2). Under the same conditions, the absorbance of thioctic acid is negligible (curve 1). Since the reagent absorbed at the wavelength of the maximum absorbance of the complex, all measurements were performed against a reagent blank.

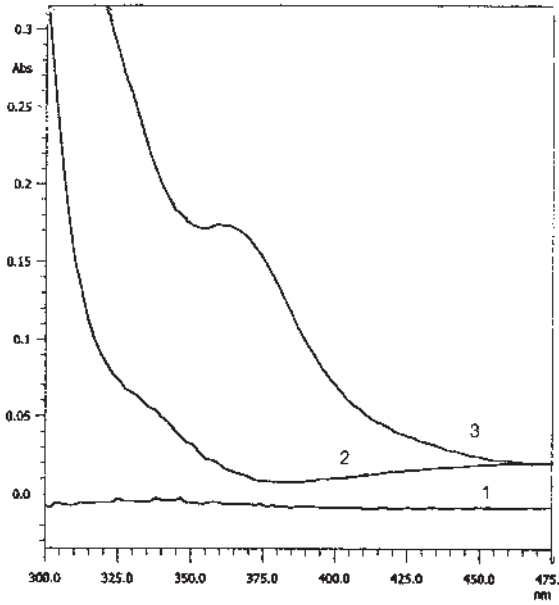

Fig. 1. Absorption spectra of thioctic acid (1), palladium(II) chloride (2) and the complex (3). c(TA) = 5×10^{-5} M; c($PdCl_2$) = 1×10^{-4} M; pH 2.2; μ = 0.2 M.

The effect of the reaction time showed that a maximum of ten minutes was necessary for the full development of the colour, which was quite stable for at least 4 h. The measurements were made at 365 nm. Although at all pH values < 4.00 spectrophotometric method could be used for the determination of thioctic acid (Fig. 2), the best results were obtained at pH 2.20, and at this pH the calibration curve was constructed. Ionic strength has little impact on the absorbance of the complex.

Physicochemical properties of the thioctic acid–Pd(II) complex

The composition of the complex was established by the continuous variations method,[17,18] and the mole ratio method.[19] These methods showed that a 1:1 complex was formed (Figs. 3–5). To determine the conditional stability constant (K'), the methods of Sommer[20] and Asmus[21] were used. These methods are based on the

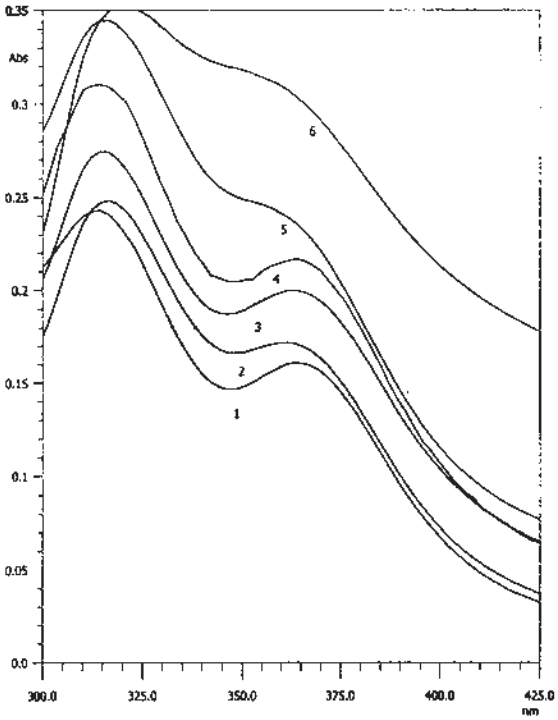

Fig. 2. The effect of pH on complex formation. c(TA) = 5×10^{-5} M; c($PdCl_2$) = 1×10^{-3} M; μ = 0.2 M; pH range 2.0 – 4.0 (curve 1–6 respectively pH: 2.20; 3.20; 3.40; 3.65; 3.80; 4.00).

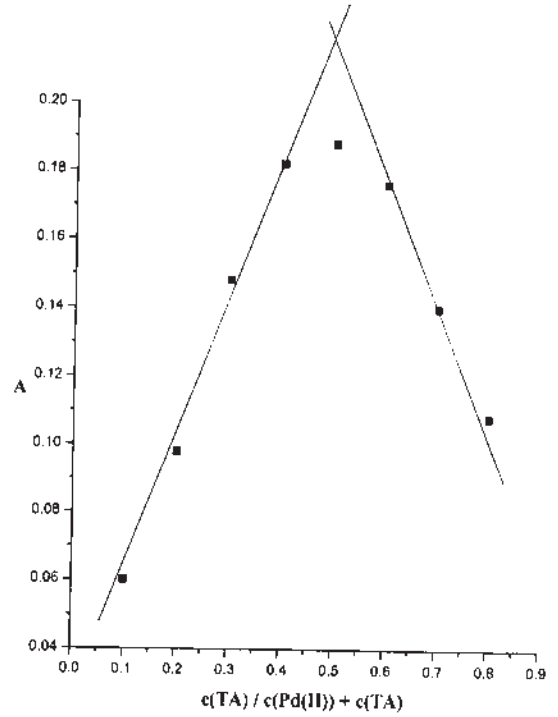

Fig. 3. Job's plot for equimolar solutions. c(TA) + c(Pd(II)) = 1×10^{-4} M; pH 3; μ = 0.2 M.

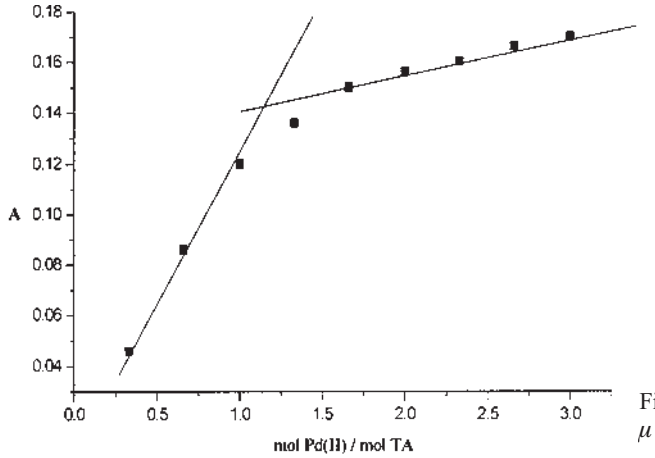

Fig. 4. Mole ratio method. pH 2.3; μ = 0.2 M.

results obtained from the Job curve of equimolar solutions (Fig. 3). The mean values of log K' obtained by the Sommer (4.50) and the Asmus (4.70) method are in good agreement.

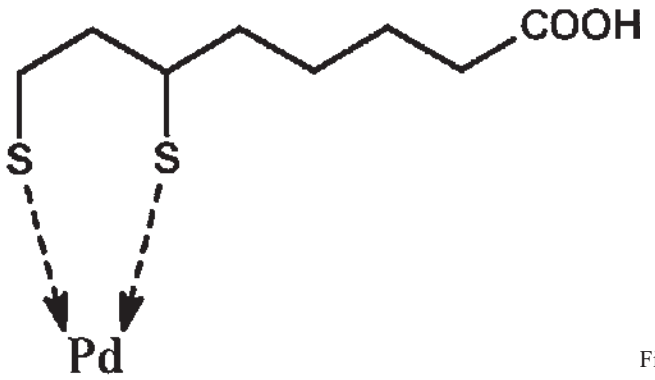

Fig. 5. Suggested structure of the complex.

Beer's law and sensitivity

Beer's law was verified in a Britton-Robinson buffer solution at pH 2.20 and λ = 365 nm. A linear relationship between the absorbance and thioctic acid concentration was obtained over the concentration range from 7.5×10^{-6} to 1×10^{-4} M; the molar absorptivity was 4.2×10^3 l mol^{-1} cm^{-1}. The regression equation was $y = 3.597 \times 10^3 \times c + 0.015$ and correlation coefficient (r) was 0.9909. The reliability of the method was checked at three different concentrations and results are summarized in the Table I. From the Table I it can be seen that the results are accurate and reproducible, and that proposed colorimetric method is suitable for the rapid determination of thioctic acid. The relative standard deviation varied from 3.10 to 3.76 % for thioctic acid (pure substance) for concentrations in the range 1.25 – 3.75 x 10^{-5} M.

TABLE I. Spectrophotometric determination of thioctic acid with palladium(II)

Taken/10^{-5} M	Found ± SD^*/10^{-5} M	RSD^{**}/%
1.25	1.251 ± 0.044	3.51
2.50	2.490 ± 0.077	3.10
3.75	3.872 ± 0.150	3.76
Taken	Found ± SD^*	RSD^{**}/%
Berlithion^R ampoules 300 mg/12 ml	299.2 ± 5.718	1.91
Thioctacid^R tablet 600 mg/tbl	597.1 ± 9.920	1.66

*Inter-assay standard deviation (n = 5); **relative standard deviation

The proposed method satisfies the analytical standards for drug determination in pharmaceutical preparations and could therefore be used for routine quality control determination of thioctic acid in injectable solutions and tablets (Table I).

Acknowledgements: The financial support for this research (Project #142071) was provided by the Ministry for Science and Environmental Protection of the Republic of Serbia.

ИЗВОД

СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ ТИОКТИНСКЕ КИСЕЛИНЕ У ВОДИ И ФАРМАЦЕУТСКИМ ПРЕПАРАТИМА

ЗАГОРКА КОРИЋАНАЦ, МИРА ЧАКАР, СЛАЂАНА ТАНАСКОВИЋ и ТАТИЈАНА ЈОВАНОВИЋ

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Описана је једноставна спектрофотометријска метода за брзо и тачно одређивање тиоктинске киселине у води, инјекционим растворима и таблетама. Спектрофотометријска мерења су извођена у Britton–Robinson-овим пуферима при различитим pH вредностима (2,00–6,00) и константној јонској сили. Састав, константа стабилности, моларна апсорптивност комплекса су одређене различитим методама. Утврђен је 1 : 1 састав награђеног комплекса и моларна апсорптивност 4.2×10^3 l mol^{-1} cm^{-1}. Релативна стандардна девијација износи од 3,10 до 3,76 %, што одговара прописаним аналитичким стандардима.

(Примљено 28. децембра 2005, ревидирано 30. маја 2006)

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