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Spectrophotometric determination of oxytetracycline in pharmaceutical preparations using sodium molybdate as analytical reagent*

M. JELIKIĆ-STANKOV,†‡ D. VESELINOVIĆ,§ D. MALEŠEV‖ and Z. RADOVIĆ‖

† *Institute of Analytical Chemistry, Faculty of Pharmacy, Belgrade, Yugoslavia*
§ *Institute of Physical Chemistry, Faculty of Science, Belgrade, Yugoslavia*
‖ *Institute of Physical Chemistry, Faculty of Pharmacy, Belgrade, Yugoslavia*

Abstract: A spectrophotometric method is proposed for the determination of oxytetracycline in pharmaceutical preparations. The method is based on the measurement of the absorbance of the molybdate–oxytetracycline complex at 404 nm (pH 5.50; μ = 0.1 M; 20°C). The composition of the complex (1:1) was determined by the application of the spectrophotometric methods of Job and Bent–French (pH 5.50; λ = 390 nm; μ = 0.1 M). The relative stability constant (K' = $10^{4.6}$) of the complex was obtained by the methods of Sommer and Nash (pH 5.50; λ = 390 nm; μ = 0.1 M; 20°C). The molar absorptivity of the complex was 9.5×10^3 l mol^{-1} cm^{-1}. Beer's law was obeyed over the concentration range 2.48–34.78 μg ml^{-1}. The relative standard deviation RSD (n = 10) was 0.27–0.39%. The method proposed can be applied to the assay of oxytetracycline in capsules. The detection limit of oxytetracycline is 2.5 μg ml^{-1}.

Keywords: *Oxytetracycline; sodium molybdate; spectrophotometric assay; pharmaceutical preparations; "Geomycine" capsules.*

Introduction

Oxytetracycline is one of the tetracycline antibiotics which react with many ions to form stable complexes [1–10]. In previous investigations the complexation reaction of tetracycline antibiotics with molybdate and wolframate ions has been studied by the application of potentiometric, conductometric and spectrophotometric methods [11–14]. The aim of the present work was to determine the composition and the stability constant of the molybdate–oxytetracycline complex, as well as to apply this complexation reaction to the assay of oxytetracycline in different pharmaceutical dosage forms by a procedure which is simpler than colorimetric methods [15, 16].

Experimental

Reagents

Oxytetracycline dihydrate (standard) was obtained from Sigma (purity 99.07%). Sodium molybdate, nitric acid, sodium hydroxide, sodium nitrate, acetic acid and

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‡To whom correspondence should be addressed.

sodium acetate were obtained from Merck. Geomycin capsules each containing 250 mg of oxytetracycline were obtained from Pliva (Zagreb). Double distilled water was used.

Solutions

For analytical purposes 1×10^{-3} M oxytetracycline was prepared freshly by dissolving an appropriate amount of oxytetracycline dihydrate (standard) in double-distilled water. 0.11 M Sodium molybdate was prepared by dissolving $Na_2MoO_4 \cdot 2H_2O$ in water; this solution was standardized gravimetrically [17]. For the analysis of Geomycine capsules, the contents of 20 capsules were mixed and a sample for the preparation of a solution containing 0.496 mg ml^{-1} of oxytetracycline was weighed and dissolved in water. This solution was filtered and diluted to 25.00 ml with water.

Acetate buffer (pH 5.50) was prepared by mixing appropriate volumes of 0.01 M sodium acetate and 0.01 M acetic acid.

The ionic strength (μ) of the solution was kept constant (0.1 M) by the addition of 2 M sodium acetate.

Adjustment of pH was carried out with solutions of nitric acid, sodium hydroxide and with acetate buffer (pH 5.50).

Apparatus

A Pye Unicam SP6-500 UV–vis spectrophotometer (Cambridge, UK) equipped with 1-cm cuvettes, an Abbot–spectrum analyser (USA) and a PHM 62 pH-meter (Copenhagen) were used.

Procedure for the calibration curve

5×10^{-3} M Sodium molybdate (2.00 ml), 2 M sodium nitrate (0.50 ml) and an aliquot (0.1–0.7 ml) of 1×10^{-3} M oxytetracycline were pipetted into a 10.00-ml volumetric flask. The pH was adjusted by the addition (2.00 ml) of acetate buffer (pH 5.50) and the solution was diluted to 10 ml with water. The absorbance of this solution at 390 nm was measured at 20 ± 0.5°C using an oxytetracycline solution of the same concentration as in the test solution (1×10^{-5}–7×10^{-5} M) as the reference.

Procedure for capsules

Aliquots of Geomycine capsule solution were treated in the same way as described for the calibration curve; the absorbance of the solutions was measured at 404 nm with reference to water, since at the latter wavelength oxytetracycline does not absorb at the concentrations investigated.

Results and Discussion

Absorption spectra

Sodium molybdate reacts with oxytetracycline in aqueous solutions at pH 3.00–8.00 to form a water-soluble complex. The absorption spectra of the complex, oxytetracycline and sodium molybdate, respectively, were recorded at 350–500 nm. Maximum absorbance of the complex occurred at 390 nm (pH 5.50) (Fig. 1, curve 1). Under the same experimental conditions oxytetracycline also absorbed radiation (Fig. 1, curve 2); therefore measurements of absorbance were performed using oxytetracycline solution as the reference. Sodium molybdate solution did not absorb at the wavelengths investigated.

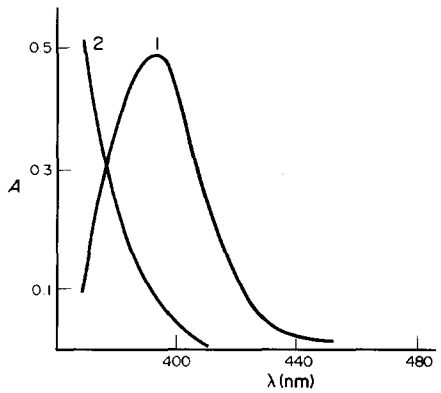

Figure 1
Absorption spectra of oxytetracycline (curve 1) and the complex (curve 2). [Oxytetracycline] = 5×10^{-5} M; [Na_2MoO_4] = 1×10^{-3} M; pH 5.50; μ = 0.1 M.

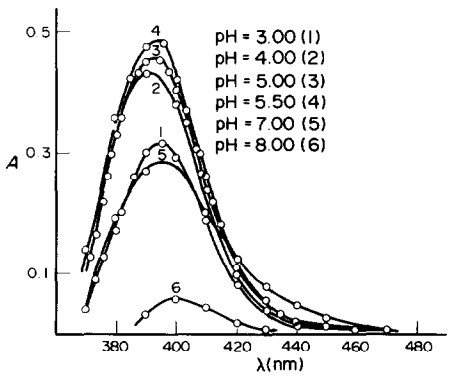

Figure 2
The effect of pH on complex formation. [Oxytetracycline] = 5×10^{-5} M; [Na_2MoO_4] = 1×10^{-3} M; μ = 0.1 M.

Effect of pH on complex formation

Absorption spectra of mixtures of 5×10^{-5} M oxytetracycline and 1×10^{-3} M sodium molybdate were recorded at pH 3.00–8.00. At pH 3.00 the complex exhibited a maximum absorbance at 400 nm; on a further increase of the pH to 7.00 the absorbance maximum appeared at 390 nm; at pH 8.00 λ_{max} was 400 nm. At pH 5.50 the absorbance of the complex was maximal. A decrease in the absorbance at higher pH is due to the dissociation of the complex and the formation of sodium molybdate (Fig. 2).

Optimum conditions for complex formation

By following the effect of the pH on the complex formation it has been established that at pH 5.50 the complex shows maximum absorbance. Investigations on the effect of sodium molybdate concentration on complex formation showed that oxytetracycline was quantitatively converted into the complex by a large excess of the reagent (1×10^{-3} M sodium molybdate; 5×10^{-5} M oxytetracycline). The colour of the complex developed instantaneously and the absorbance remained unchanged for 40 min.

The effect of ionic strength (0.1–0.7 M) on complex formation was insignificant.

Composition of the complex

The stoichiometric ratio of components in the complex was determined by the application of Job's method of continuous variations [18, 19]. The curve had a maximum at a molar fraction $x_{max} = 0.5$, which indicated the formation of a 1:1 complex (Fig. 3).

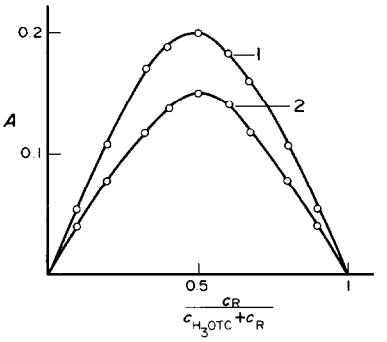

Figure 3
Job's curves of equimolar solutions at 390 nm (curve 1) and 410 nm (curve 2). [Oxytetracycline] + $[Na_2MoO_4] = 2 \times 10^{-4}$ M; pH 5.50; $\mu = 0.1$ M. Oxytetracycline = H_3OTC.

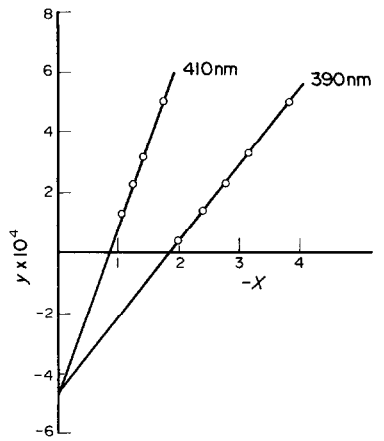

Figure 4
Nash's method. [Oxytetracycline] = 1.6×10^{-4} M; $[Na_2MoO_4] = 2 \times 10^{-5}$–$8 \times 10^{-5}$ M; pH 5.50; μ = 0.1 M.

The composition of the complex was confirmed by Nash's method [20]. There was a linear dependence for $y = f(-x)$ (Fig. 4), where $y = 1/c_{\text{molybdate}}$ and $x = A_o(A_o - A)$ (where A_o is the absorbance of oxytetracycline and A the absorbance of the complex). The composition of the complex was also determined by the method of Bent–French [21]. The results obtained ($p = 1.28$–0.94; $q = 1.03$–0.94) indicated a 1:1 stoichiometric ratio of the components.

Relative stability constant of the complex

From potentiometric investigations it has been established that the complexation of molybdate with oxytetracycline proceeds as for other tetracycline analogues [13]. In this reaction, the molybdate ion is transformed into the oxide MoO_3 which reacts with oxytetracycline. Since no data are reported on the reaction:

$$MoO_4^{2-} + 2\,H^+ \rightleftarrows MoO_3 + H_2O,$$

the calculated stability constant is relative and specific.

By applying the method of Sommer [22] on the basis of results obtained by Job's method for the composition of the complex, the relative stability constant of the complex has been determined (Table 1). The stability constant was calculated also by the application of Nash's method [20], the value of the constant being obtained as the

Table 1
Relative stability constants of the oxytetracycline–molybdate complex*

Sommer's method					
(nm)	log K'	log K'_{min}	log K'_{max}	SD†	RSD‡ (%)
390	4.53	4.50	4.55	0.02	0.44
410	4.49	4.47	4.51	0.01	0.22
Nash's method					
(nm)	log K'				
390	4.63	4.62 ± 0.01			
410	4.60				

*Conditions: pH 5.50; μ = 0.1 M; I = 20 ± 0.5°C.
†SD for standard deviation (n = 10).
RSD = relative standard deviation.

negative intercept on the y axis (Table 1). The values for the relative stability constant of the complex, obtained by two different methods, are in good agreement.

Quantification of Beer's law

Linear dependence of the complex absorbance on oxytetracycline concentration was established for 2.50–34.78 μg ml^{-1}. The molar absorptivity of the complex was 9.5×10^3 l mol^{-1} cm^{-1}. The regression equation was $y = 15.23x - 3.208 \times 10^{-3}$ and the correlation coefficient (r = 0.9997) indicated good linearity. The lower sensitivity limit of the method was 2.5 μg ml^{-1} of oxytetracycline. The precision of the method was determined at three different concentrations of the antibiotic (Table 2). The RSD was 0.30–1.02% for oxytetracycline concentrations of 0.00487–0.01491 mg ml^{-1}.

Analysis of pharmaceuticals

The method was applied to the determination of oxytetracycline in Geomycine capsules (Pliva); accurate and reproducible results were obtained.

The main advantage of the method is its simplicity compared with that of previous colorimetric procedures [15, 16]. No heating is required and only a slightly acid medium is used; this medium does not cause degradation of oxytetracycline. In addition, the detection limit of the method is lower than that of other colorimetric methods for the assay of oxytetracycline [15, 16].

Table 2
Spectrophotometric determination of oxytetracycline in capsules using sodium molybdate*

Oxytetracycline dihydrate		0.00497 mg ml^{-1}	0.00994 mg ml^{-1}	0.01491 mg ml^{-1}
Found	$\bar{x}$	0.00501	0.00996	0.01494
	x_{min}	0.00499	0.00992	0.01488
	x_{max}	0.00504	0.01000	0.01500
	SD†	0.00002	0.00003	0.00004
	RSD‡ (%)	0.39	0.30	0.27

*Conditions: λ = 404 nm; pH 5.50.
†SD = inter-assay standard deviation (n = 10).
‡RSD = relative standard deviation.

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