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Short Communication

Analytical investigations of β-lactam antibiotics in pharmaceutical preparations — II. Spectrophotometric determination of cephalexin, cephradine, ampicillin and amoxycillin using copper(II) acetate as a complexing agent

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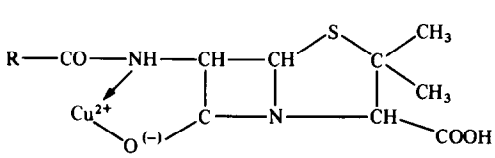
Introduction

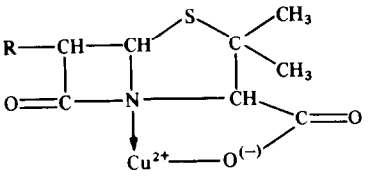
The pharmacological interest and the increasing production, uses and consumption of β-lactam antibiotics, especially of cephalexin(I), cephradine(II), ampicillin(III) and amoxycillin(IV), are the principal reasons that a considerable number of various procedures for their assay have been published [1–8] in addition to official methods. A spectrophotometric procedure for the determination of the β-lactam antibiotics using paramolybdate anion has been reported previously by the present author [9].

The complexation of Cu(II) ions with β-lactam antibiotics was studied more than 20 years ago. Cressman *et al.* stated [10, 11] that the effect of Cu(II) on the penicillins was to promote their degradation to co-ordination complexes of Cu(II) and the corresponding penicilloic acids. Complexation was assumed to occur between Cu(II) and the intact penicillins followed by a rate-limiting hydrolysis of the complex into the corresponding penicilloic acid-Cu(II) complex.

The same research team later studied [12] the mechanism of this complexation and concluded that Cu(II) interacts catalytically with penicillins through the formation of a five-membered chelate of the following type:



Results of the work of Weis *et al.* [13, 14] and Johnson *et al.* [15] led to the possibility that the following type of chelate also exists:



If this chelate was formed it would be "non-catalytic", as shown by kinetic experiments with penicillanic acid. Such a chelate would, however, decrease the amount of free Cu(II) available for chelation at the catalytic site.

The reaction scheme which would conform with the overall assumption of complexation followed by hydrolysis is:

$$\text{Cu(II)} + \text{penicillin} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Cu(II)} - \text{penicillin}$$

$$\text{Cu(II)} - \text{penicillin} + OH^- \xrightarrow[k_3]{} \text{Cu(II)} - \text{penicilloic acid}$$

$$\text{Cu(II)} - \text{penicillin} + H_2O \xrightarrow[k_4]{} \text{Cu(II)} - \text{penicilloic acid} + H_3^+O$$

On the other hand, a number of investigators [16–18], studied complex formation and redox reactions between Cu(II) and D-penicillamine; other studies have been conducted on the formation, behaviour and the determination of stability constants of the complexes of penicillins with various metal cations [19, 20].

It should also be mentioned that the *British Pharmacopoeia* includes a colour test for the identification of cephalexin based on the formation of a complex between this substance and Cu(II) [21]. Saha [22] recently described a spectrophotometric method for the determination of benzylpenicillin in pharmaceutical preparations using Cu(II) as a chromogenic reagent.

In the present study a simple, accurate, convenient and reproducible spectrophotometric method for the determination of I, II, III and IV using Cu(II) acetate as complexing agent is reported. The suggested procedure is easily applied to the determination of the drugs in pure form and in pharmaceutical preparations (capsules, injections); the results are in good agreement with those obtained by official methods [24, 25].

Experimental

Apparatus

A Hitachi model 100-80, double-beam ratio recording spectrophotometric system, with 10.0-mm quartz cells was used for the absorbance measurements during the development of this procedure.

A WTW pH-meter model pH 522, with a precision combined electrode E56, was used for the pH measurements.

An Ultrathermostat model NBS (Gebrüder Haake K.G.) was used.

Reagents

Cephalexin, was obtained from Chemische Fabric Schweizerhall, Basel.

Cephradine was provided by Savalon, Italy.

Ampicillin trihydrate was obtained from Ankerfarm, Italy.

Ampicillin sodium was obtained from Liessa, Spain.

Amoxycillin trihydrate was obtained from Gema, Spain.

Cupric(II) acetate RPE-ACS and anhydrous sodium acetate RPE-ACS were obtained from Farmitalia, Carlo Erba, Italy.

Solutions

Standard solutions of I, II, III and IV were freshly prepared by dissolving the appropriate amount from each one in 0.2 M sodium acetate to form a 1.0% (m/v) solution expressed as anhydrous substance. Each millilitre of the solution contained 10 mg of antibiotic.

Solution of copper(II) acetate was prepared by dissolving the appropriate amounts of the corresponding reagent in distilled water to form a 1.0% (m/v) solution expressed as the anhydrous salt. Each millilitre of the solution contained 10 mg of copper(II) acetate.

Sodium acetate solution 0.2 M was prepared by dissolving 16.4 g of sodium acetate in distilled water and diluting to 1 l.

Solutions of I, II, III and IV in 0.2 M sodium acetate and a solution of copper(II) acetate in distilled water with the same molarity (0.03 M) were used only for the determination of the molar ratios of the resulting complexes by application of the method of continuous variations.

Recommended procedures

For antibiotic substances. A volume of standard solution of the antibiotic (2.0–10 ml containing 20–100 mg of antibiotic) accurately measured was transferred to a 50-ml volumetric flask. An equal volume of Cu(II) acetate solution accurately measured, was added to the same flask and the mixture was diluted to volume with 0.2 M sodium acetate. The solution was mixed by shaking and was kept for 5 min at 20 ± 0.5°C; the absorbance was measured within 10 min at the wavelength of maximum absorbance against 0.2 M sodium acetate as the blank.

For injections of ampicillin sodium. A sample of the injection containing about 500 mg of ampicillin sodium, accurately weighed, was transferred quantitatively into a 100-ml volumetric flask and dissolved in about 60 ml of distilled water; the solution was diluted to volume with the same solvent and mixed by shaking. 10.0 ml of this (volume equivalent to 50 mg of ampicillin sodium) was transferred by pipette into a 50-ml volumetric flask, and 5.0 ml of the Cu(II) acetate solution was added.

The mixture was diluted with 0.2 M sodium acetate to volume and mixed. The flask was kept for 5 min at 20 ± 0.5°C and the absorbance of the emerald-green solution was measured within 10 min at 640 nm against 0.2 M sodium acetate as the blank.

Calculation of the concentration of ampicillin sodium in the solution was effected by reference to the calibration graph.

For capsules and tablets. A quantity of the mixed contents of 20 capsules or of 20 powdered tablets (equivalent to approximately 500 mg of antibiotic) was accurately weighed and was transferred to a 100-ml volumetric flask.

Methanol (60 ml) *pro analysi* was added and the flask was shaken vigorously for at least 15 min. The flask was filled to the mark with methanol (analytical reagent grade). The extraction did not affect the stability of the ampicillin and other antibiotics.

After mixing, the contents of the flask were filtered through a fast filter-paper. The first fraction (15–20 ml) of the filtrate was rejected and the following 10.0 ml of filtrate (equivalent to approximately 50 mg of antibiotic) was transferred by pipette into a 50-ml volumetric flask. The solution was evaporated quickly to dryness under vacuum (5–10 mm Hg and <40°C). After evaporation, about 20 ml of 0.2 M sodium acetate was added to dissolve the residue. Copper(II) acetate solution (5.0 ml) was added to the same flask. The mixture was diluted to volume with 0.2 M sodium acetate and mixed. The flask was kept for 5 min at 20 ± 0.5°C.

The absorbance of the coloured solution was measured within 10 min at the wavelength of maximum absorbance against 0.2 M sodium acetate as the blank.

The calculation of the concentration of antibiotic in the measured solution was effected by reference to the appropriate calibration graph.

Results and Discussion

Absorption spectra

The absorption spectra of the complex species that were formed between I, II, III and IV and Cu(II) were measured against 0.2 M sodium acetate as the blank in the range 520–750 nm and are illustrated in Fig. 1.

The λ_{max}, the molar absorptivity (ϵ), the colour of the complex species, the maximum time of the stability of the colour, the optimum pH for formation of the complex and the molar ratios of these complex species in solution, which were studied spectrophotometrically by the method of continuous variations, are given in Table 1.

Calibration graphs

Calibration graphs (concentration against absorbance) were traced for each of the complex species between I, II, III, IV and Cu(II) at the corresponding optimum pH and wavelength of maximum absorbance.

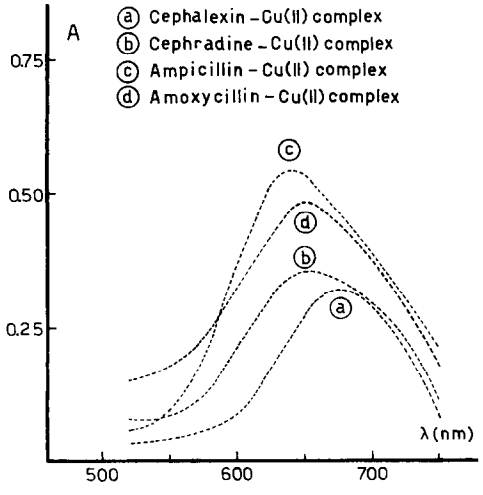


Figure 1
Absorption spectra of β-lactam antibiotics — Cu(II) complexes. Antibiotic concentration, 1.0 mg ml^{-1}. pH = 6.55 ± 0.05. Blank: 0.2 M sodium acetate solution.

Table 1
Spectral and optical characteristics and constants of β-lactam antibiotics–Cu complexes

	Cephalexin	Cephradine	Ampicillin	Amoxycillin
λ_{max}	675 nm	650 nm	640 nm	650 nm
ϵ (Molar absorptivity)	1.12×10^2	1.25×10^2	1.89×10^2	1.69×10^2
Colour	Brilliant green	Blue	Emerald green	Blue-green
Maximum time of stablility of the colour*	20 min	20 min	20 min	20 min
Optimum pH	6.55 ± 0.05	6.55 ± 0.05	6.55 ± 0.05	6.55 ± 0.05
Molar ratio (M:L)	2:1	2:1	2:1	2:1

*Temperature = 20 ± 0.5°C.

Conformity with the Beer–Lambert law was observed over the concentration range 0.25–3 mg antibiotic per ml of the measured solution; the greatest accuracy was attained for solutions containing 5×10^2–2×10^3 μg antibiotic per ml.

For cephalexin, the regression equation for absorbance (y) against concentration in mg ml^{-1} (x) was $y = 0.3205 \times 10^{-3}x + 0.001$ ($N = 8$); standard error (SE) in gradient = 5.0×10^{-9}. The relative standard deviation (RSD) at 1 mg ml^{-1} = 1.9% ($N = 10$).

For cephradine, the regression equation was $y = 0.3632 \times 10^{-3}x - 0.0032$ ($N = 8$); SE in gradient = 3.9×10^{-9}. RSD at 1 mg ml^{-1} = 3.1% ($N = 10$).

For ampicillin, the regression equation was $y = 0.5518 \times 10^{-3}x - 0.0108$ ($N = 8$); SE in gradient = 1.76×10^{-8}. RSD at 1 mg ml^{-1} = 0.8% ($N = 10$).

For amoxycillin, the regression equation was $y = 0.4668 \times 10^{-3}x + 0.0072$ ($N = 8$); SE in gradient = 9.9×10^{-9}. RSD at 1 mg ml^{-1} = 0.9% ($N = 10$).

Effect of pH

The effect of pH on the formation of the complex species M_2L, (where M = Cu^{2+} and L = I or II or III or IV) was investigated. Figure 2 shows the results for the pH range 4.5–7.0. The study of the effect of pH higher than 7.0 was considered as purposeless because Cu(II) hydroxide was formed followed by hydrolysis and turbidity of the measured solution. For solutions of pH lower than 4.5 the penicillins in an acetate medium containing a trace amount of Cu(II) undergo a controlled degradation to

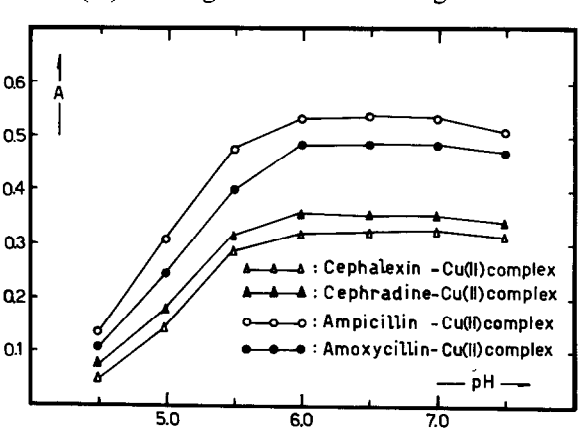


Figure 2
Effect of pH on the absorbance in the corresponding λ_{max} of the Cu(II)-β-lactam antibiotics complexes. (Concentration of the β-lactam antibiotic = 1.0 mg ml^{-1}.)

Table 2
Determination of β-lactam antibiotics by the proposed procedure compared with official methods

Substance or formulated product	Proposed procedure Amount taken or declared mg	Mean* found mg	Recovery* %	Standard deviation %	Official methods Amount taken or declared mg	Mean* found mg	Recovery* %	Standard deviation %	Official method
Cephalexin	500	492	98.4	0.61	500	502	100.4	0.76	B.P.†
Keflex (Eli Lilly) Capsules	500	510	102.0	0.61	500	516	103.2	0.96	p. 86–87
Cephradine	500	500	100.0	0.25	500	510	102.0	0.21	B.P.†
Velosef (Squibb) Capsules	500	505	101.0	0.31	500	515	103.0	0.31	p. 89
Ampicillin trihydrate	500	495	99.0	0.36	500	497	99.4	0.37	U.S.P.‡
Pentrexyl (Bristol) Capsules	500	500	100.0	0.44	500	508	101.6	0.79	p. 59–63
Ampicillin sodium sterile	500	490	98.0	0.42	500	496	99.2	0.69	U.S.P.‡
Penbritin (Beecham) Injection	500	518	103.6	0.48	500	520	104.0	0.88	p. 59–63
Amoxicillin trihydrate	500	498	99.6	0.29	500	500	100.0	0.61	U.S.P.‡
Amoxil (Beecham) Capsules	500	510	102.0	0.81	500	518	103.6	0.45	p. 56–57
Mean recovery %			100.36%				101.64%		

*Means of five determinations.
†B.P.: *British Pharmacopoeia* (1980) [24].
‡U.S.P.: *United States Pharmacopeia* XXI [25].

penicillenic acid [23]. The optimum pH range (6.55 ± 0.05) was obtained by use of 0.2 M sodium acetate.

Effect of excipients

Excipients usually added in the preparation of tablets such as lactose, sugar, starch, powdered acacia, talc, magnesium stearate, polyvinylpyrrolidone and sometimes colloidal silicon dioxide (especially for the filling of the capsules) did not interfere with the results.

On the other hand, in the author's opinion, extraction with methanol of the antibiotics from the contents of capsules (if necessary) and from powdered tablets eliminates completely any improbable and undesirable effect caused by these excipients.

Comparison of assay with official methods

In order to confirm its usefulness the proposed method was applied to the determination of the β-lactam antibiotics as the substances and in commonly used pharmaceutical formulations. Table 2 gives the results obtained by simultaneous application of the proposed procedure and an official method which was used in each case for the comparison of analytical results.

Comparison between the sets of the results shows that there is no significant difference between the recommended procedure and the official methods of determination.

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