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Vanadophosphoric Acid as a Modified Reagent for the Spectrophotometric Determination of Certain Cephalosporins and their Dosage Forms

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Summary. Vanadophosphoric acid in acidic medium is proposed as a modified reagent for the spectrophotometric determination of cephalexin, cephaprine sodium, cefazolin sodium, and cefotaxime in pure samples and in pharmaceutical preparations. The method is based on acid hydrolysis of cephalosporins and subsequent oxidation with vanadophosphoric acid. The resulting solution exhibits maximum absorption at about 516 nm. The effects of reaction conditions were investigated. *Lambert-Beer*'s law was obeyed over a concentration range of about 0.4–45 $\mu g \cdot cm^{-3}$. For more accurate results, *Ringbom* optimum concentration ranges were obtained, and the molar absorptivities and *Sandell* sensitivities were derived. The proposed method was applied to the determination of the drugs in pharmaceutical formulations; the results demonstrated that the proposed method is as accurate, pecise, and reproducible as the official methods.

Keywords. Vanadophosphoric acid; Antibiotics; UV/Vis spectroscopy; Pharmaceutical formulations.

Introduction

The chemistry of cephalosporins has been widely explored because of their extensive medical applications. The available visible spectrophotometric methods for the determination of cephalosporins are based on acetylation of cephalexin with acetic anhydride in aqueous solution at *pH* 11.5[1]. The formation of ethylene blue after hydrolysis in sodium hydroxide solution to give hydrogen sulfide [2,3], charge-transfer complex formation with iodine [4], 2,3-dichloro-5,6-dicyano-*p*-benzoquinone [4], and chromotrope 2B and 2R have also been used [5]. In addition, several analytical procedures are available in the literature for the analysis of cephalosporins, *e.g.* fluorometry [6–8], chemiluminescence [9], titrimetry [10, 11], polarography [12], stripping voltammetry [13, 14], HPLC [15, 16], and iodometric methods [17, 18].

Salts of vanadium(V) have been used as oxidizing agents in spectophotometric determinations of β-lactam antibiotics (semi-synthetic penicillins, cephalosporins)

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[19–21] and other compounds of pharmacological interest. In this work, however, we did not use a salt but rather a heteropoly acid of vanadium(V), vanadophosphoric acid (*VPA*). With this modification, the absorbance of the measured solution remains constant for a longer period (at least 6 h) after the heat treatment, and the molar absorptivity is higher (and, therefore, the *Sandell* sensitivity is decreased).

The aim of the present work is to hydrolyze cephalosporins in sulfuric acid media and to treat them with vanadophosphoric acid as an oxidizing agent in order to develop a modified spectrophotometric method for their determination in raw material and in pharmaceutical formulations.

Results and Discussion

A detailed investigation of the reaction between the hydrolysis products of cephalexin (**1**), cephaprine sodium (**2**), cefazolin sodium (**3**), and cefotaxime (**4**) and vanadophosphoric acid showed that they are oxidized in acidic medium. The coloured product had a considerable absorbance in the region of interest. Accordingly, the absorbance measurements were carried out against a reagent blank prepared in the same way. Several parameters as acidity, oxidant concentration, time, and temperature were optimized to achieve high sensitivity, low blank reading, and high stability.

Effect of acidity

A detailed study of the reaction in various concentrations of different mineral acids (HCl, H_2SO_4, and H_3PO_4) showed that sulfuric acid in the concentration ranges 1.5–3.0, 1.5–2.5, 1.5–2.8, and 1.5–3.0 *M* in the final assay solution was necessary for complete colour development for **1**, **2**, **3**, and **4**. A 2.0 *M* sulfuric acid solution in the final volume was selected for all further studies, since the results are highly concordant at this concentration.

Effect of oxidant concentration

To fix the optimum reagent concentration for complete red colour development in a total volume of 25 cm^3, the concentration of vanadophosphoric acid was varied. The optimum amount of vanadophosphoric acid was found to be 5.0 cm^3 of 0.10% (w/v) in all subsequent studies (Fig. 1).

Effect of order of additions

From experiments in which the reagents were added in all possible sequences it was concluded that the maximum absorbance is attained according to the following order: cephalosporin/sulfuric acid/vanadophosphoric acid.

Effect of time and temperature

The maximum absorbance reading of the red coloured species was obtained after 5.0 min of mixing the reactants at 25±2°C. The absorbance remained constant for a

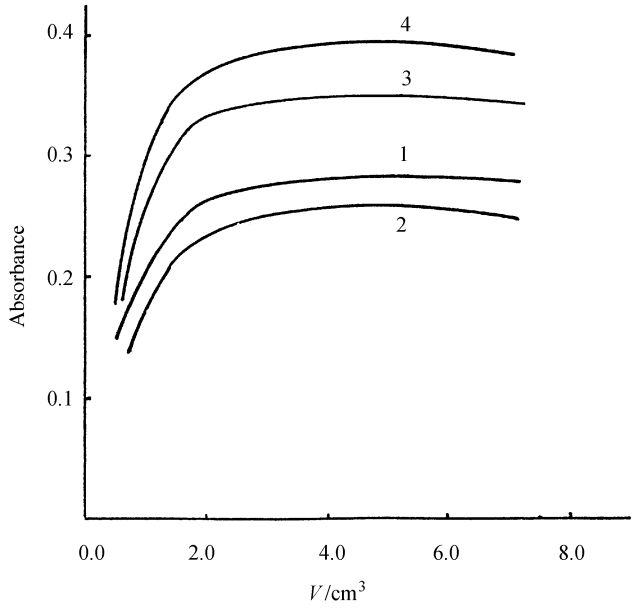

Fig. 1. Effect of vanadophosphoric acid concentration on the absorbance at λ_{max} of the red product for 18 $\mu g \cdot cm^{-3}$ of drug **1**–**4**

period of 12, 15, 9.0, and 6.0 h for **1**, **2**, **3**, and **4**, above which the absorbance gradually decreased at a rate of 3.0% for every 5.0 min. The red colour intensity remained unaltered in the temperature range of 20–45°C for **1** and **3**, whereas for **2** and **4** it remained unchanged in the temperature range of 20–60°C, above which the absorbance gradually decreased.

Under the above optimum conditions, the spectra of the red coloured species showed an absorption band at $\lambda_{max} = 515$, 512, 518, and 523 nm for **1**, **2**, **3**, and **4** (Fig. 2). Vanadophosphoric acid and the cephalosporins under consideration do not absorb in the range of 400–700 nm, and therefore the analytical conditions are excellent.

Analytical validity

Beer's law was obeyed in the concentration range of 0.4–42.0, 0.4–45.0, 0.4–36.0, and 0.4–33.0 $\mu g \cdot cm^{-3}$ for **1**, **2**, **3**, and **4**. The optimum concentration ranges evaluated by *Ringbom*'s method [22] are 1.5–40.0, 1.5–42.5, 1.0–34.0, and 1.0–31.0 $\mu g \cdot cm^{-3}$, respectively. The molar absorpitivities and *Sandell* sensitivities [23] (in $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ and $\mu g \cdot cm^{-2}$) were 5.28×10^3 and 0.0692 for **1**, 5.83×10^3 and 0.0763 for **2**, 8.47×10^3 and 0.0562 for **3**, and 9.01×10^3 and 0.0505 for **4**. The regression equations calculated from the calibration graphs were $A = 0.007 + 0.0146C$ for **1**, $A = 0.009 + 0.0131C$ for **2**, $A = 0.005 + 0.0178C$ for **3**, and $A = 0.008 + 0.020C$ for **4**, where A is the absorbance and C is the concentration ($\mu g \cdot cm^{-3}$). The correlation coefficients were 0.9988, 0.9995, 0.9998, and 0.9996 for **1**, **2**, **3**, and **4**, respectively. To test the validity of the proposed method it was applied to authentic samples of the examined drugs. In order to determine the

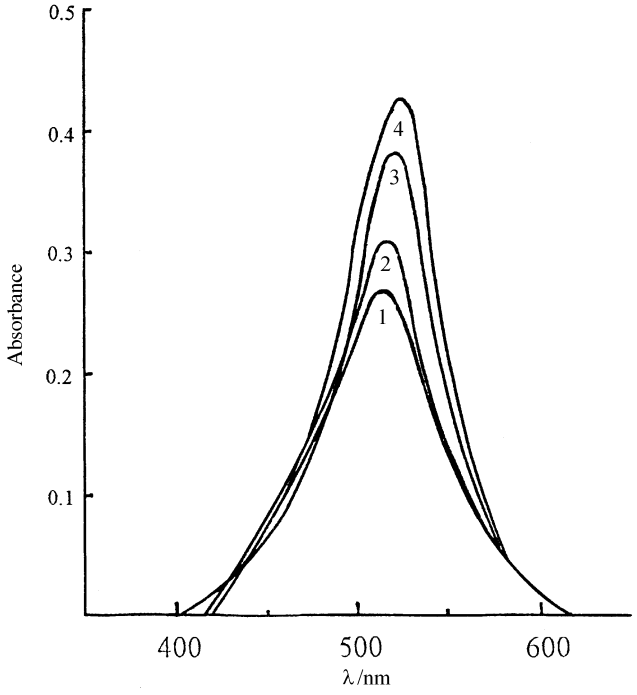

Fig. 2. Absorption spectra of the red coloured product of 20 μg · cm^{-3} of drugs **1–4** using 5 cm^3 0.1% vanadophosphoric acid in 2 *M* H_2SO_4

accuracy and precision of the method, solutions containing five different concentrations of the drug were prepared and analyzed in quintuplicate. The relative errors ($\leq$1.60) and relative standard deviations ($\leq$1.81) can be considered as satisfactory for the concentration levels examined. Thus results given in Table 1 show that the method is accurate and precise.

Interferences

Several pharmaceutical preparations of cephalosporins are associated with flavouring agents, diluents, and excipients such as maltose, sucrose, lactose, starch, citric acid, tartaric acid, benzoic acid, sodium bicarbonate, magnesium stearate, and reserpin. In preliminary experiments, these compounds were tested with vanadophosphoric acid under the same conditions as for pure samples of the cephalosporins and found not to interfere up to a 30–50 fold excess. Although penicillin V, penicillin G, tetracycline, and oxytetracycline interfere in the determination of cephalosporins, this poses no problem due to their absence in pharmaceutical formulations.

Analytical applications

The proposed method was evaluated by analyzing some commercial formulations of cephalosporins and comparing the results with those obtained using the official

Table 1. Analysis of authentic samples of the drugs **1–4** by the proposed (prop.) and by official (off.) methods [17, 18]

Sample Concentration ($\mu g \cdot cm^{-3}$)	Found Concentration[a]/$\mu g \cdot cm^{-3}$							
	1		2		3		4	
	prop.	off.	prop.	off.	prop.	off.	prop.	off.
7.0	7.05 (±0.41)	6.90	6.97 (±0.66)	6.88	6.95 (±0.46)	7.10	7.05 (±0.58)	7.15
14	13.28 (±0.67)	13.75	14.05 (±0.37)	13.70	13.90 (±0.33)	14.30	14.30 (±0.43)	13.75
21	20.80 (±0.73)	21.30	21.15 (±0.44)	20.75	21.15 (±0.59)	20.70	20.80 (±0.79)	21.40
28	28.25 (±0.56)	27.60	27.85 (±0.58)	28.35	28.20 (±0.68)	27.50	28.25 (±0.94)	28.40
35	3.50 (±0.39)	35.70	35.30 (±0.92)	34.54	34.80 (±0.82)	36.00	–	34.30
42	41.60 (±0.88)	42.50	42.40 (±0.71)	42.60	42.60 (±0.67)	43.00	–	41.60

[a] Each result is the average of six determinations

method (titration of excess iodine with sodium thiosulfate) [17, 18]. The performance of the recommended method was assessed by calculation of *t*-values [24]. Mean values of 0.983, 1.126, 0.866, and 0.937 for **1**, **2**, **3**, and **4** showed the absence of any systematic error in the method. The corresponding tabulated *t*-value for five degrees of freedom and a 95% confidence level was 2.57. Therefore, it can be concluded that there is no significant difference between the proposed and the official methods [17, 18] (cf. Table 2). However, the proposed method has the advantage of ease of operation, convenience, small sample/reagent consumption, and it can be used as a stability indicating method.

Chemistry of the coloured species

The formation of the coloured products is based on the acid hydrolysis of the cephalosporins with sulfuric acid, followed by the reduction of vanadium(V) in the vanadophosphoric acid to vanadium (IV) under specific experimental conditions that yield a blue coloured product (vanadium (IV)) at first. This product reacts rapidly with one of the degradation products of the cephalosporins affording a red coloured complex with absorptions at 515, 512, 518, and 523 nm for **1**, **2**, **3**, and **4**. The stoichiometric ratio of the formed complex was established by the molar ratio and continuous variation methods. The results of both methods suggested the formation of an 1:1 complex. The logarithmic stability constants of an 1:1 complexes were calculated from *Harvey* and *Manning*'s equation [25] using the data obtained from both methods. The values of stability constants were found to be 4.05, 4.87, 4.58, and 4.33 for **1**, **2**, **3**, and **4**.

Table 2. Analysis of dosage forms containing cephalosporins **1**–4 by the proposed (prop.) and by official (off.) methods [17,18]

Drug	Formulation	Manufacturer	Content ($mg/5\,cm^3$)	Found[e] ($mg/5\,cm^3$)				
				prop ±SD(%)	RSD(%)	off. ±SD(%)	RSD%	t[f]
1	Neocef syrup	Nile[a]	250	247 ±0.86	1.38	245 ±1.13	1.84	1.217
	Neocef capsule	Nile	250	248 ±067	1.19	254 ±1.04	1.69	1.323
	Neocef capsule	Nile	500	504 ±0.93	1.43	493 ±1.08	1.56	1.015
2	Cefatrexyl vail	Bristol Myer[b]	500	502 ±0.71	1.71	495 ±0.95	1.42	1.167
			1000	999 ±0.55	1.08	993 ±0.88	1.37	0.981
			2000	2005 ±0.49	1.22	1990 ±0.77	1.51	1.239
3	Kefzol vail	EIPICO[c]	500	499 ±0.38	1.03	505 ±0.69	1.63	1.415
			1000	998 ±0.42	1.16	994 ±0.91	1.75	1.439
4	Claforan vail	Hoechst[d]	1000	995 ±0.75	1.34	991 ±1.016	1.89	1.517

[a] Nile: The Nile Company for Pharmaceuticals and Chemical Industries, Egypt; [b] Bristol Myer: Bristol Myer Company, Squibb, Egypt; [c] EIPICO: Egyptian International Pharmaceutical Industries Company, Egypt, in cooperation with F. Hoffmann-La Roche Ltd., Basle, Switzerland; [d] Hoechst: Hoechst Orient, Egypt, under licence of Hoechst AG, Frankfurt, Germany; [e] average of six determinations; [f]: calculated t-value (The theoretical value is 2.57 for five degrees of freedom and 95% confidence limits)

Experimental

Apparatus

A Perkin-Elmer Lambda 3B spectrophotometer with matched 10 mm quartz cells was used for all absorbance measurements during the development of the procedure.

Materials

Pure drug samples were kindly provided from several manufactures. The purity of the compounds was checked and established by the official method [20]. Standard cephalosporin solutions ($100\,\mu g \cdot cm^{-3}$) were prepared by dissolving 0.01 g substance (accurately weighed) in water and diluting to $100\,cm^3$ in a calibrated flask. The working solutions were obtained by further dilution.

Reagents

All chemicals and reagents used were of analytical grade. Doubly distilled demineralized H_2O was used throughout. Vanadophosphoric acid solution (0.10% (w/v)) was prepared by dissolving 0.10 g in H_2O and diluting to $100\,cm^3$ in a calibrated flask. The H_2SO_4 acid stock solution (4.0 *M*) was prepared in the usual way.

General procedure

Various aliquots containing 10–1050 μg **1**, 10–1125 μg **2**, 10–900 μg **3** or 10–825 μg **4** were transferred into a series of 25 cm^3 calibrated flasks. 12.5 cm^3 of 4.0 *M* H_2SO_4 acid was added to each flask so that the final concentration was 2.0 *M*. After adding 5.0 cm^3 of 0.10% vanadophosphoric acid to each flask, the solutions were made up to the mark with water. The absorbance was measured after 5.0 min of mixing at 515, 512, 518, and 523 nm for **1**, **2**, **3**, and **4** against a reagent blank prepared in the same manner. The cephalosporin concentration was then deduced from an appropriate calibration graph prepared previously.

Procedure for capsules and syrup

A quantity of the mixed contents of 10 capsules or a suitable amount of powdered suspension equivalent to 0.01 g of the drug was dissolved in H_2O and diluted to 100 cm^3 in a calibrated flask. An appropriate volume of the filtrate was transferred into a 25 cm^3 calibrated flask and treated as above.

Procedure for vails

An accurately weighed sample of a vail equivalent to 0.01 g of the drug was dissolved in H_2O and diluted to 100 cm^3 in a calibrated flask. An appropriate volume was transferred into a 25 cm^3 calibrated flask and treated as above.

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