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Determination of penicillin V potassium in pharmaceuticals and spiked human urine by chemiluminescence

Research Article

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Abstract: A simple and selective method for penicillin V potassium (PVK) determination by chemiluminescence (CL) was developed. Oxidation of PVK by alkaline hydrogen peroxide produces CL, which is greatly enhanced by N, N-dimethyl formamide (DMF) and N-cetyl-N,N,N-trimethylammonium bromide (CTMAB). Optimum conditions were established using luminometry. There is a linear relationship between the chemiluminescent peak height and the amount of PVK within the range 0.5 - 129.5 mg L^{-1}, with a detection limit of 0.2 mg L^{-1}. The coefficient of variation was 1.2% for 40 mg L^{-1} PVK solution (n = 7). The method is very simple, has high sensitivity and good selectivity, and is usable for process control. It was successfully utilized for the determination of PVK in pharmaceuticals and spiked human urine.

Keywords: *Chemiluminescence • Penicillin V potassium • N, N-dimethyl formamide • Human urine • Pharmaceuticals*

1. Introduction

Penicillin V potassium (PVK) is used to treat dental, cardiac, middle ear, dermal, and respiratory tract infections, as well as rheumatic fever and scarlet fever [1]. PVK determination is important in biotechnological production process control and antibiotic preparation quality control. A literature survey reveals several methods for PVK determination including spectrophotometry [2-4], HPLC [5-7], liquid chromatography [8], and capillary electrophoresis [9].

Chemiluminescent (CL) reactions have potential for a great variety of analytical applications thanks to their high sensitivity, wide linear range, simplicity, and inexpensive instrumentation [10-12]. In the past two decades trace determinations by CL have markedly increased [13-18]. Such methods have recently been suggested for determination of structurally related antibiotics such as amoxicillin, penicillin G, and many other aminopenicillin compounds [19-24].

The absence of a reported PVK CL determination sparked the authors' interest in this problem. Moreover, our experiments revealed that the CL intensity was a linear function of the concentration of PVK within the range 0.5 - 129.5 mg L^{-1}. Here, we have proposed a novel, fast, and simple CL method for determination of PVK in biological fluids, which can also be used as an alternative method for quality control of pharmaceutical products.

2. Experimental Procedures

2.1. Apparatus

CL was monitored by a Junior LB 9509 luminometer (Berthold Technologies, Germany). The time resolution was 1 s and CL intensity was reported in relative arbitrary units (a.u.). Experiments were carried out under mechanical stirring at room temperature (ca. 21°C). All pH measurements were made with a digital pH meter (Metrohm 744). ALBET® DP 150 125 filter papers were used for filtration.

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2.2. Material

All chemicals were of analytical grade and supplied by Merck, except PVK which was purchased from Fluka. All were used as received. Hydrogen peroxide solutions were prepared by diluting appropriate amounts of 30% H_2O_2 to 5 mL. The H_2O_2 solutions were prepared and consumed freshly. Stock PVK (0.02 M) was prepared in doubly distilled water and stored in a refrigerator. Stock 2% (w/v) *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTMAB) was prepared in doubly distilled water. Na_2HPO_4/NaOH buffer (pH 12) was prepared and used for analytical determinations.

2.3. Procedure

CL reactions were carried out in a 12 mm × 47 mm test tube placed in the luminometer sample holder. A 2.0 mLportion of solution containing 1 × 10^{-2} M pH 12 phosphate buffer, 2% (w/v) *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTMAB), 1.5 × 10^{-2} M ethylenediaminetetraacetic acid disodium salt (Na_2H_2Y), and 30% (v/v *N, N*-dimethyl formamide (DMF) (reagent 1) was added into the test tube. Then, 1 mL of 5% (w/v) H_2O_2 (reagent 2) was injected into the test tube to initiate the CL reaction. The lid was closed and the progress of the reaction was continuously monitored on a computer connected to the luminometer. After approximately 100 s, when the baseline signal reached a minimum, varying volumes of the sample or standard PVK solution (providing 0.5 - 129.5 mg L^{-1} PVK in the final solution) were injected into the test tube. Final solutions with PVK concentrations 0.5 - 20 mg L^{-1} were prepared by injecting 2 - 80 µL of 0.002 M (10-fold diluted stock solution) PVK solution, while the concentration range of 20 - 129.5 mg L^{-1} was obtained by injecting 8 - 50 µL of 0.02 M (stock) PVK solution. The cover of the reaction chamber was then immediately closed and the progress of the CL reaction continuously monitored.

3. Results and Discussion

Preliminary experiments indicated that CL occurs upon injection of PVK into alkaline H_2O_2–DMF-CTMAB solution, and emission intensity is dependent on the antibiotic concentration.

3.1. Kinetics

One of the important characteristics of a CL reaction is its kinetic profile. Fig. 1 shows the CL intensity–time profile for the reaction obtained with 40 mg L^{-1} PVK, 30% (v/v) DMF, 2% (w/v) CTMAB, 1.5 × 10^{-2} M Na_2H_2Y,

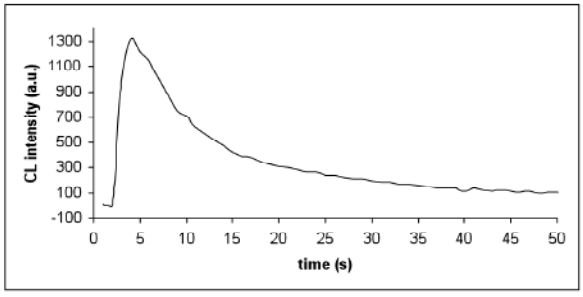

Figure 1. CL time profile. Reagent 1: 2% (w/v) CTMAB, 30% (v/v) DMF, pH 12; Reagent 2: 5% (w/v) H_2O_2; 40 mg L^{-1} PVK

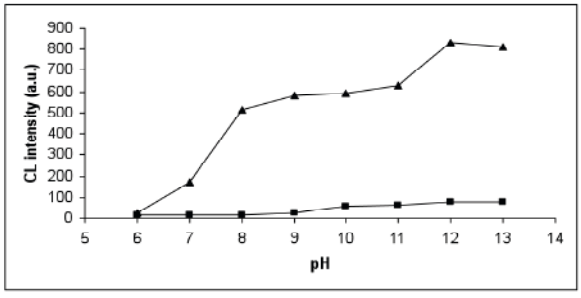

Figure 2. The effect of pH in the absence (■) and in the presence (▲) of 40 mg L^{-1} PVK. Reagent 1: 1% (w/v) CTMAB, 30% (v/v) DMF. Reagent 2: 5% (w/v) H_2O_2

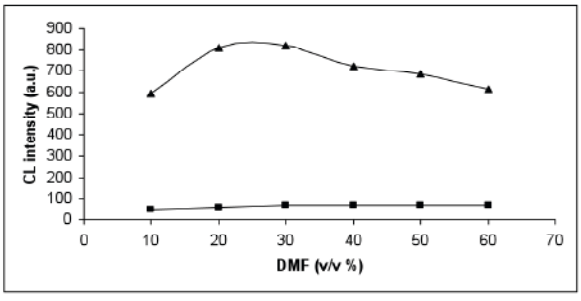

Figure 3. The effect of DMF (reagent 1) in the absence (■) and in the presence (▲) of 40 mg L^{-1} PVK. Reagent 1: 1% (w/v) CTMAB, pH 12, and DMF. Reagent 2: 5% (w/v) H_2O_2

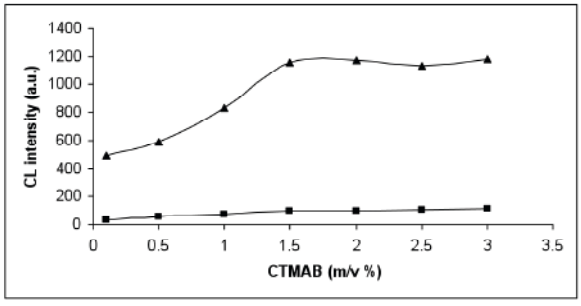

Figure 4. The effect of CTMAB concentration (reagent 1) in the absence (■) and in the presence (▲) of 40 mg L^{-1} PVK. Reagent 1: 30% (v/v) DMF, pH 12, and CTMAB. Reagent 2: 5% (w/v) H_2O_2

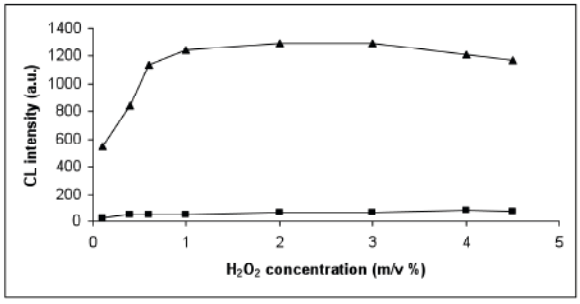

Figure 5. The effect of H_2O_2 concentration (reagent 1) in the absence (■) and in the presence (▲) of 40 mg L^{-1} PVK. Reagent 1: 2% (w/v) CTMAB, 30% (v/v) DMF, pH 12. Reagent 2: H_2O_2

was observed 3 - 5 seconds after the injection of PVK and then the signal decayed. The maximum was used as the analytical signal for the rest of the study.

3.2. Optimization

Several variables were optimized to maximize the sensitivity. Fig. 2 shows the effect of pH on the CL intensity. The CL reaction is critically dependent on pH and occurs only above pH 6; pH 12 provides the highest CL intensity. The working pH was therefore adjusted with pH 12 phosphate buffer. The buffer concentration did not affect the CL intensity up to 0.1 M. Fig. 3 shows that CL intensity increases with increasing DMF concentration and remains fairly constant over the range 20 - 30%, then decreases at higher concentrations. Therefore, 30% (v/v) DMF was taken as the optimum value. Fig. 4 shows the effect of CTMAB concentration; 2% (w/v) was chosen. Fig. 5 shows that CL intensity increases with increasing H_2O_2 concentration up to 1% and reaches a plateau at higher concentrations. Hydroxyl and superoxide radicals, which play critical roles in the CL system, are produced by radical decomposition of H_2O_2 , but H_2O_2 is also a powerful ·OH scavenger. It seems that at higher than 3%, its radical scavenging counteracts its positive role as the radical source for the reaction. Therefore, 2% hydrogen peroxide was taken as the optimum value.

3.3. Analytical parameters

Under optimum reaction conditions, peak height vs. the amount of PVK (mg L^{-1}) was plotted for a series of seven standard solutions. The plot was linear over the analyte range 0.5-129.5 mg L^{-1}. The corresponding linear regression equation is: I = 81.27 + 31.28 X (r^2 = 0.994), where I = peak height, and X = PVK concentration (mg L^{-1}). The standard deviations of the calibration line slope and intercept were 0.41 and 8.53, respectively. Using the IUPAC definition of the limit of detection (c_{LOD} = analyte concentration giving a signal equal to blank signal + three standard deviations of the blank signal) the estimated c_{LOD} was 0.2 mg L^{-1}. The coefficient of variation (CV) calculated for 7 replicate determinations of 40 mg L^{-1} PVK was 1.2%.

3.4. Interferences

Interference due to some common excipients, complexing agents, and substances generally found in urine was studied. Analyses were carried out for a fixed concentration of penicillin V (40 mg L^{-1}) and increasing interferent concentrations. The tolerance level was defined as the interferent level leading to an error not exceeding ± 5% analyte. In the hope of reducing cation interference, 1.5×10^{-2} M Na_2H_2Y was added throughout the rest of the experiments, and all interference effect investigations were also carried out in the presence of Na_2H_2Y (with the exception of glycine, sodium citrate, and Na_2H_2Y). Results are summarized in Table 1.

Table 1. Tolerable concentration ratios with respect to 1.0×10^{-4} M (40 mg L^{-1}) PVK for some interfering species

Species	Tolerable concentration ratio
Na^+, K^+, Cl^-	1450
Glycine	350
Na_2H_2Y	250
lactose, sucrose	200
sodium citrate	150
urea, Al^{3+}, Cr^{3+}	100
Co^{2+}	50
Mg^{2+}, Ba^{2+}, Cd^{2+}	40
Ca^{2+}, Ni^{2+}, Sr^{2+}	30
Cu^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}	20
starch[a]	25

[a] *concentration of starch and PVK in mg L^{-1}*

According to manufacturers' specifications, common excipients used in PVK tablets are lactose, magnesium stearate, povidone, starch, stearic acid (octadecanoic acid), hydroxypropyl methylcellulose, polyethylene glycol, talc, titanium dioxide, dibasic calcium phosphate, microcrystalline cellulose, and sodium citrate. Fortunately, most of these ingredients are water-insoluble and can easily be separated *via* filtration. The rest of the excipients do not interfere. We conclude that the CL method provides selective determination of PVK in these formulations.

3.5. Analytical applications

3.5.1. Determination of PVK in pharmaceutical tablets

The method was applied to the determination of PVK in tablets from two different manufacturers (MATER Co., LTD. and Dava Pharmaceuticals Inc.). For each brand, ten 250 mg tablets were powdered, homogenized and weighed to obtain the average

weight. A portion of powder equivalent to approximately 400 mg penicillin V potassium was accurately weighed and dissolved in about 30 mL water. The resulting solution was filtered into a 50 mL volumetric flask and brought to volume with added beaker washings. The resultant sample solution was appropriately diluted with water and analyzed according to the procedure described in the experimental section. The accuracy of the analyses was validated *via* recovery tests. Results are summarized in Table 2.

3.5.2. Determination of PVK in spiked urine

To demonstrate satisfactory selectivity and sensitivity for the measurement of penicillin in biological samples, human urine spiked with exactly known amounts of the antibiotic was analyzed. First, the urine pH was increased to about 8 with 6 M NaOH and filtered to remove precipitates. A standard addition method was used to avoid possible matrix effects. The results are summarized in Table 3.

Table 3. Determination of PVK in spiked human urine

Sample	Added concentration (mg L^{-1})	Amount found[a] (mg L^{-1})
1	10.0	9.8 ± 0.1
2	50.0	50.1 ± 0.2

[a] *Mean of five replicate determinations*

4. Conclusion

Penicillin V potassium oxidation by hydrogen peroxide causes strong CL emission. The experimental conditions were optimized to develop a useful analytical method for determination of penicillin V. Its applicability was validated by successful analysis of the antibiotic in pharmaceutical preparations and in spiked human urine.

Table 2. Determination of PVK in pharmaceutical formulations

Sample	Nominal content (mg/tablet)	Amount found[a] (mg/tablet)	Amount added (mg/tablet)	Amount found [a] (mg)	Recovery [a] (%)
tablet 1	250	252 ± 3	—	—	101 ± 1 [b]
			30	281 ± 2	97 ± 3 [c]
			50	303 ± 4	102 ± 2 [c]
tablet 2	250	254 ± 3	—	—	102 ± 1 [b]
			30	285 ± 2	103 ± 5 [c]
			50	302 ± 2	96 ± 3 [c]

[a] *Mean of five replicate determinations ± standard deviation*
[b] *recovery compared to the nominal content*
[c] *recovery compared to the added amount*

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