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Liquid chromatographic determination of residual hydrogen peroxide in pharmaceutical excipients using platinum and wired enzyme electrodes

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Abstract

Hydrogen peroxide (H_2O_2) is a chemically reactive reagent that can oxidize and degrade many pharmaceutical compounds under normal conditions. Unfortunately, H_2O_2 is often introduced into pharmaceutical excipients during manufacturing and it may significantly affect the chemical stability of drugs in formulations. Thus, a sensitive analytical method for determination of residual H_2O_2 in excipients is of importance in formulation development and product quality control. A liquid chromatographic system with a dual channel electrochemical detector (LCEC) was equipped with either a platinum electrode or a wired peroxidase electrode for determination of H_2O_2. The excipient (0.1 g) was dissolved in 10 ml of mobile phase and 5 μl of the dissolved solution was directly injected. The chromatographic run time for each sample was 1 min with a detection limit of 10 ng/ml (S/N = 5) using the platinum electrode and 1 ng/ml (S/N = 5) using the wired enzyme coated electrode, respectively. The peak purity was assured by comparing the peak ratios at different potentials for both the standard and the samples. The H_2O_2 levels in different batches of PVP, PEG, and other surfactants from different manufacturers were determined and the values ranged from 0 to 244 ppm. The LCEC method is exceptionally fast, accurate and convenient for quantitation of low levels of residual H_2O_2 in pharmaceutical formulation excipients.

Keywords: Hydrogen peroxide; Electrochemical detection; Formulation; Excipients

1. Introduction

Drug chemical stability can be decreased by impurities that exist in formulation excipients. Hydrogen peroxide (H_2O_2) is one of the impurities that can oxidize drugs. One of our experiments revealed that a drug was significantly degraded due to the presence of polyvinylpyrrolidone (PVP), an excipient widely used in pharmaceutical formulations. Further studies indicated that the presence of residual H_2O_2 in the PVP was the cause of drug oxidation. The determination of residual H_2O_2 in excipients is of importance in formulation development, drug stability study and

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excipient quality control. This report shows that PVP, polyethylene glycol (PEG) and other common surfactants that are often used in pharmaceutical formulations contain appreciable amount of residual H_2O_2.

There are many analytical methods for the determination of H_2O_2. The titration methods reported in the US Pharmacopoeia [1] and the British Pharmacopoeia [2] are for the determination of concentrated H_2O_2 solutions (30%) and topical solutions (3%). The titration methods are not sufficiently sensitive for determination of residual H_2O_2 in excipients. In the past decade, the behavior of H_2O_2 on platinum and gold electrodes was extensively studied in physiological buffers [3,4]. Carbon paste electrode [5], sol–gel-biosensor [6], silica gel immobilized electrode [7], reagentless enzyme coated film electrode [8], bacteria-based biosensor [9], enzymatically modified printed film electrode [10], nickeocene-mediating sensor [11], mediatorless HRP-modified carbon paste electrode [12] and catalase biosensor [13] were reported for biosensing glucose and H_2O_2. Besides biosensors, chemiluminescence and fluorometric methods were reported for determination of H_2O_2 after derivatization [14–16]. To the best of our knowledge, the determination of residual H_2O_2 in pharmaceutical excipients has not been reported.

In this study, LCEC with either the platinum electrode or the wired horseradish peroxidase (HRP) electrode was explored for the determination of low levels of residual H_2O_2 in water soluble excipients.

2. Experimental

2.1. Reagents and chemicals

Hydrogen peroxide (30%) was obtained from Mallinckrodt and stored at 4 °C. HPLC grade water (OmniSolv) was obtained from EM Science. Potassium phosphate monobasic was obtained from Mallinckrodt and ethylenediamine tetraacetic acid, disodium salt dihydrate (EDTA) was obtained from Aldrich. HPLC certified disposable filters (0.45 μm, 25 mm discs, Acrodisc, CR PTFE) were products of Gelman Sciences. All chemicals were analytical reagent grade.

The sample diluent solution was the same as the mobile phase. Since two different mobile phase systems were used for two different types of electrodes, the diluent solution was changed accordingly. The sample diluent solution was used as the washing solution for flushing the injector needle of the autosampler.

A peroxidase electrode kit was purchased from Bioanalytical Systems (BAS) (p/n: MF-2095). The kit includes a HRP polymer coating solution, electrode surfactant solution, and a cross-flow glassy carbon dual electrode.

2.2. Liquid chromatographic conditions

The system consisted of a LC system and an electrochemical detector. The LC system consisted of a BAS PM-80 pump (BAS), a Sample Sentinel Auto-sampler (BAS), and a Luna LC column (C18(2), 4.6 × 100 mm, 5 μm, Phenomenex) at ambient temperature.

The mobile phase was a 50 mM KH_2PO_4 buffer solution adjusted to pH 8.0 with 8 M NaOH solution for use with the platinum electrode and the pH was 5.5 for the wired enzyme electrode. The flow rate of the mobile phase was 1 ml/min. Each injection volume was 5 μl and the run time was 1 min for each injection.

2.3. Electrochemical detection conditions

A dual channel BAS LC-4C Amperometric Detector was utilized in this study. The potential at the platinum electrode was at +500 mV versus Ag/AgCl for oxidation. The 3 mm diameter platinum electrode was obtained from BAS. Both the platinum electrode and the wired enzyme electrode were dual cross-flow electrodes. When the wired enzyme electrode was used, the potential was at +100 mV versus Ag/AgCl for reduction. The detector filter was set at 0.1 Hz.

When the dual channel detector was used for assessment of interference, the dual platinum electrode and the dual wired enzyme electrode were placed in parallel in the flow. Two different potentials were applied on the two parallel electro-

des. The peak area ratios on the two channels were recorded.

2.4. Preparation of electrodes

A polished and cleaned platinum electrode was stabilized before use. The electrode was placed on the system at a potential of +500 mV versus Ag/AgCl for at least 1–2 h or overnight. A low current background and reproducible peak response were obtained after stabilization.

The wired HRP enzyme electrode was made by coating the HRP polymer on a polished and cleaned glassy carbon electrode. The surfactant solution (0.5 μl) in the peroxidase electrode kit was dispensed on the surface of the electrode and this was dried for about 10 min. The peroxidase/polymer solution (0.5 μl) was dispensed on the surfactant treated surface. The electrode was stored at room temperature overnight to dry and cure. The prepared enzyme electrode was able to function for about 2 weeks. Fig. 1 is a photograph of the platinum, the glassy carbon and the wired HRP enzyme electrodes.

2.5. Sample preparation procedures

The formulation excipient (0.10 g) was dissolved in 10 ml of the mobile phase and vortexed if needed. If the excipient formed a suspension and could not be completely dissolved in the mobile phase, the suspension was centrifuged. A 0.45 μm disposable filter was used to filter the sample if the supernatant was not clear after centrifugation. A 5 μl sample of the solution was directly injected into the LCEC system.

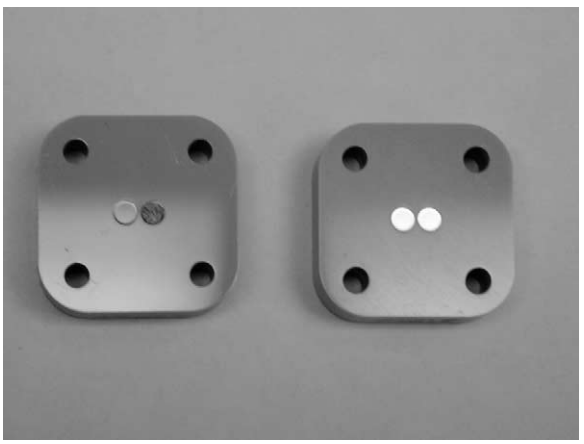

Fig. 1. Photograph of electrodes: Right: dual platinum electrode Left: dual glassy carbon electrode; the left side is the naked glassy carbon electrode, the right side is coated with the HRP enzyme.

3. Results and discussion

Hydrogen peroxide can be directly oxidized at a platinum working electrode [3,17]:

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$$

The hydrodynamic voltammogram suggested that H_2O_2 was well oxidized at +500 mV versus Ag/AgCl. Potentials higher than +500 mV resulted in a higher background current. The pH of the mobile phase affected both the detection sensitivity and the background current. To examine the relationship between pH and response, 5 μl of a 30 μg/ml H_2O_2 solution was repeatedly injected into the LCEC system with variation of the mobile phase pH from 3 to 9. The results show that the higher the pH of the mobile phase, the higher the current response on the platinum electrode (Fig. 2). A mobile phase pH of 8 was selected for high sensitivity and an acceptably low background current. Activation of the working platinum electrode was needed when the peak size

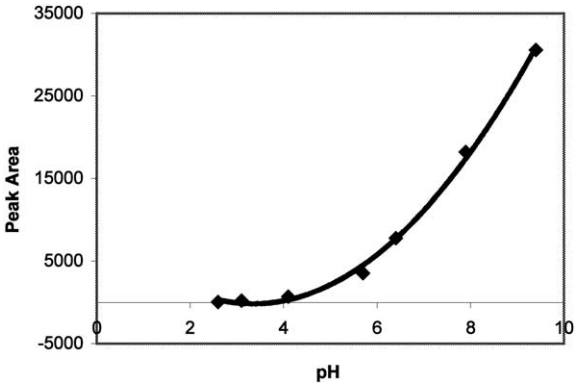

Fig. 2. The relationship between the mobile phase pH and the peak area when using the Pt electrode. 5 μl of 30 μg/ml of hydrogen peroxide was repeatedly injected while the pH of the mobile phase was changed and the peak areas were measured.

of a standard solution dropped by more than 50%. In this study, polishing the electrode once a week was sufficient to keep the electrode activated. After polishing, the platinum electrode was placed in the cell at +500 mV for a few hours or overnight for stabilization. The detection limit was 10 ng/ml using the platinum electrode. The regression coefficient, r^2, was 0.9992 with concentrations from 0.05 to 7.5 μg/ml (5 μl was injected) and $r^2 = 0.9888$ from 0.05 to 30 μg/ml.

The accuracy and precision data using the platinum electrode is shown in Table 1. A known amount of H_2O_2 was spiked into the diluent solutions and the H_2O_2 concentration in the spiked solutions was determined. The results show that the analytical recoveries were between 95.0 and 100.7% using the platinum electrode. The recoveries were obtained by using the sample diluent without the excipients because of the difficulty of homogeneously spiking hydrogen peroxide into the solid excipients and the lack of blank excipients without residual hydrogen peroxide. Hydrogen peroxide is highly soluble and, therefore, complete dissolution of hydrogen peroxide should occur. The R.S.D. was 1.8–4.3%. Fig. 3 shows a chromatogram of 14 injections of a 300 ng/ml H_2O_2 solution within 40 min using the platinum electrode that had been continuously used for 3 days. The R.S.D. of the 14 peak areas was 1.4%.

Hydrogen peroxide can be electrochemically reduced at a mediated or wired peroxidase electrode [18,19], and the electrode was used in determination of acetylcholine, glucose, and lactate in microdialysates [20,21]. The redox scheme is illustrated in Fig. 4. HRP is covalently linked to the redox hydrogel coated on the surface of a glassy carbon electrode. The redox polymer, derived from poly(vinylpyridine) partly complexed to $[Os(bpy)_2Cl]^{2+/3+}$ redox centers, electrically 'wires' the HRP enzyme to the glassy carbon electrode. The wired enzyme electrode is based on monitoring the electro-reduction of the oxidized mediator generated by an enzyme-catalyzed reaction. This results in high sensitivity and provides highly efficient electron transfer. Based on a glassy carbon electrode, the wired enzyme electrode stabilizes much faster than the platinum electrode, usually around 10 min. The electrode was operated at +100 mV (vs. Ag/AgCl) for the reduction of H_2O_2. Lower potentials were not suitable because they caused interference due to the reduction of oxygen present in the samples. A pH 5.5 mobile phase was chosen because of the high enzyme activity, high electrochemical reduction sensitivity and low background current.

For the method using the wired peroxidase electrode, r^2 was 1.00 for determination of H_2O_2 in the concentration range 0.01–1.20 μg/ml and r^2 was 0.9971 in the concentration range 0.01–6 μg/ml. The detection limit was 1 ng/ml with a signal to noise ratio of 5. This detection limit was equivalent to 0.1 ppm of H_2O_2 in the excipients based on a

Table 1
Analytical precision and recovery of H_2O_2 using the platinum electrode

Spiked (ng/ml)	1200	600	300	150	75
Determined (ng/ml)	1077	611	308	146	75
	1193	611	299	143	72
	1236	615	288	145	70
	1251	587	290	144	70
	1201	583	295	139	71
	1235	589	297	141	72
	1252	606	291	143	73
	1195	595	288	144	69
	1247	594	294	139	71
	1202	606	296	141	71
Average	1209	600	294	143	71
SD	52.1	11.3	6.2	2.5	1.7
R.S.D. (%)	4.3	1.9	2.1	1.8	2.4
Analytical recovery (%)	100.7	99.9	98.1	95.0	95.0

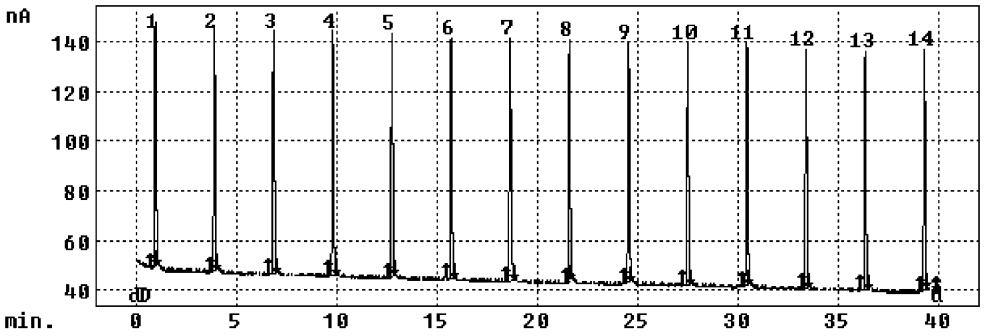

Fig. 3. Peak reproducibility using the platinum electrode. 5 μl of a 300 ng/ml standard solution was repeatedly injected 14 times to test the precision of the platinum electrode response. The R.S.D. of the peak areas was 1.4%.

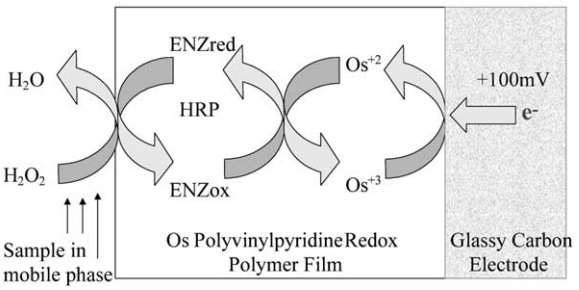

Fig. 4. Redox cycle for wired HRP electrode. Abbreviations in the figure: ENZred, reduced form of the enzyme; ENZox, oxidized form of the enzyme; HRP, horseradish peroxidase.

100 fold dilution factor during sample preparation. The analytical recoveries were between 97.9 and 98.9% with an R.S.D. of 0.53–0.74%. Fig. 5 shows typical chromatograms of H_2O_2 in various pharmaceutical excipients using the wired enzyme electrode. The freshly coated enzyme electrode could be used for about 13 days. By this time, the current response was about 50% of the original value, and the electrode was re-coated. Compared with the platinum electrode, the wire enzyme electrode was more sensitive and selective; however, the peak showed slight tailing probably because the coating layer on the electrode slowed the diffusion speed of H_2O_2.

Under the two chromatographic conditions using two different electrodes, H_2O_2 was rapidly eluted with the solvent similar to flow injection analysis. To minimize the solvent peak that was co-eluted with H_2O_2, the excipient sample was diluted 100 times with the mobile phase. As a result, the solvent peak was negligible and did not influence the accuracy of the analytical results. The reverse phase column served to provide backpressure for proper operation of the pump. The column also served to retain small amounts of hydrophobic impurities if present in the excipients. Periodically washing the column (twice a month) with 60% acetonitrile regenerated the column activity.

Dual channel electrochemical detectors can be used for voltammetric characterization of eluting compounds [22–24]. In this report, the peak purity was confirmed by comparing the peak area ratios at different potentials for both the standard and the sample. Two different potentials were applied on a dual electrode placed in parallel. Comparison of the current–potential response of an H_2O_2 standard and the excipient samples provided confirmation of the peak purity. A change in the peak area ratio was an indication of interference due to co-elution. Table 2 shows that the peak area ratios of the H_2O_2 standard and the excipients tested were in good agreement when the two wired peroxidase electrodes were applied at 0 mV and at +100 mV. This suggests that there was no interference due to co-elution with H_2O_2. However, when the platinum electrode was used with polysorbate 80, the peak area ratio obtained at +500 as compared with +400 mV was significantly different from the peak area ratio of the H_2O_2 standard (Table 3). This difference suggested that there was an electrochemically active interference co-eluting with H_2O_2 when polysorbate 80 was present. Changing the two potentials to +500 and

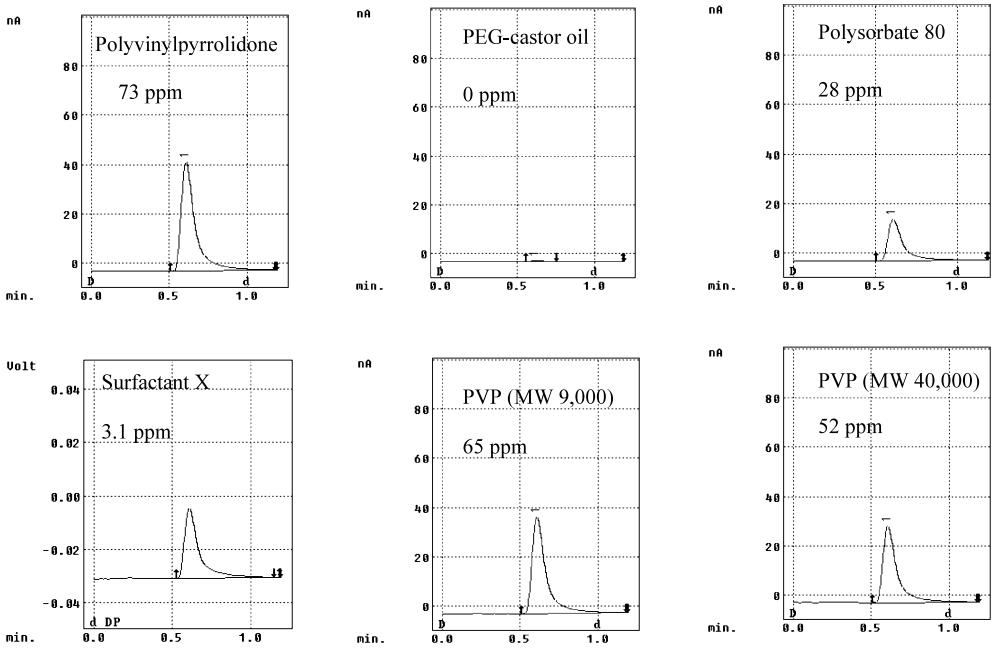

Fig. 5. Typical chromatograms of hydrogen peroxide in excipients when the wired enzyme electrode was used.

Table 2
H_2O_2 peak area ratios using the dual wired enzyme electrode

Excipient	Peak area ratio (+100/0 mV)
H_2O_2 standard	0.93
PVP (MW 10 000)	0.90
PVP (MW 150 000)	0.95
Polysorbate 80	0.92
PVP (MW 9000)	0.90
PEG 400 NF	0.95

Table 3
H_2O_2 peak area ratios using the dual platinum electrode

Excipient	+500/+850 mV	+500/+400 mV
H_2O_2 standard	0.68	1.5
PVP (MW 10 000)	0.67	1.5
PVP (MW 150 000)	0.65	1.6
PVP (MW 9000)	0.66	1.5
PEG 400 NF	0.65	1.4
Polysorbate 80	0.76	1.0

to +850 mV further confirmed the difference in the peak area ratio as compared with that of the H_2O_2 standard. In contrast, other excipients showed essentially the same ratio, indicating that determination of residual H_2O_2 in excipients using the platinum electrode was not interfered by these excipients. No interference was found when the enzyme electrode was used. *t*-Butyl peroxide, a representative alkyl peroxide, was tested and it showed no response in this assay. This result indicates that alkyl peroxides do not interfere with the determination of H_2O_2 using this method.

The H_2O_2 concentrations in common pharmaceutical excipients were determined by this method as shown in Table 4 (the H_2O_2 concentrations varied from 0 to 244 ppm). The high concentration of H_2O_2 observed in these common excipients may have a significant impact on the chemical stability of drugs when the excipients are used in drug formulations. These results indicate that a screen for H_2O_2 in excipients could be useful in selecting vendors or batches of excipients with low levels of residual H_2O_2.

Table 4
Hydrogen peroxide content in pharmaceutical excipients

Excipient	Manufacturer	Batch	H_2O_2 content (ppm)
PEG 400	A	A-1	39
PEG 400	B	B-1	16
PEG 400	B	B-2	4.9
PEG 400	B	B-3	9.6
PEG 400	C	C-1	59
PEG 400	C	C-2	1.7
PEG 400	D	D-1	25
PVP (MW 4000)	E	E-1	27
PVP (MW 9000)	E	E-2	65
PVP (MW 50 000)	E	E-3	188
PVP (MW 150 000)	F	F-1	150
PVP (MW 40 000)	B	B-4	110
Poly-XL PVP	G	G-1	58
PVP (MW 40 000)	B	B-5	52
Polyvinylpyrrolidone-A	G	G-2	244
Polyvinylpyrrolidone	H	H-1	73
PEG–castor oil	E	E-4	0
PEG–castor oil	E	E-5	2.9
PEG–castor oil (hydrogenated)	E	E-6	1.0
Surfactant X	I	I-1	3.1
Polysorbate	H	H-2	28
Polysorbate	H	H-3	18

4. Conclusions

The liquid chromatographic method using electrochemical detection with either platinum electrode or wired enzyme electrode is accurate and sensitive for determination of low levels (>1 ng/ml) of residual H_2O_2 in pharmaceutical excipients. The wired HRP enzyme electrode is more sensitive and selective than the platinum electrode, however, the later is easier to use. Polysorbate 80 interferes with the determination of H_2O_2 when the platinum electrode is used, but not when the wired HRP enzyme electrode is used.

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