Evaluation of Hydroperoxides in Common Pharmaceutical Excipients

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ABSTRACT: While the physical properties of pharmaceutical excipients have been well characterized, impurities that may influence the chemical stability of formulated drug product have not been well studied. In this work, the hydroperoxide (HPO) impurity levels of common pharmaceutical excipients are measured and presented for both soluble and insoluble excipients. Povidone, polysorbate 80 (PS80), polyethylene glycol (PEG) 400, and hydroxypropyl cellulose (HPC) were found to contain substantial concentrations of HPOs with significant lot-to-lot and manufacturer-to-manufacturer variation. Much lower HPO levels were found in the common fillers, like microcrystalline cellulose and lactose, and in high molecular weight PEG, medium chain glyceride (MCG), and poloxamer. The findings are discussed within the context of HPO-mediated oxidation and formulating drug substance sensitive to oxidation. Of the four excipients with substantial HPO levels, povidone, PEG 400, and HPC contain a mixture of hydrogen peroxide and organic HPOs while PS80 contains predominantly organic HPOs. The implications of these findings are discussed with respect to the known manufacturing processes and chemistry of HPO reactivity and degradation kinetics. Defining critical HPO limits for excipients should be driven by the chemistry of a specific drug substance or product and can only be defined within this context. J Pharm Sci 96:106–116, 2007

Keywords: chemical stability; physicochemical properties; oxidation; formulation; excipients

INTRODUCTION

Excipients in pharmaceutical dosage forms are increasingly viewed as important contributors to the overall properties of the dosage form. As such, excipient characterization efforts have become more prevalent. The physical properties of excipients have received the most attention, and the topics of excipient functionality testing and multisource excipient equivalence have been largely discussed in this context.[1,2] However, the chemical impurity profiles of excipients have not, in general, received similar attention. Excipient chemical impurity profiles can be very important in influencing the long-term chemical stability performance of the formulated drug product, particularly if an oxidatively sensitive drug is being formulated.

Oxidative degradation leads to the loss of drug potency over time and may challenge formulation development, reduce shelf life for a drug candidate, prolong development, and delay time to market. In this context, trace level hydroperoxide (HPO) impurities can play a major role. HPOs can

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be an "organic" hydroperoxide (ROOH), where R is a carbon atom, or hydrogen peroxide (H_2O_2). HPOs can be catalytically decomposed by trace levels of transition metals (such as iron(III)) as well as by heat and light to give peroxy and alcoxyl radicals. These radicals can initiate chain propagation of peroxy radicals, which subsequently react with and degrade oxidatively sensitive drug substances (single-electron transfer).[3–5] Direct reaction of HPOs with nucleophilic groups (two-electron transfer) such as amines and thio-ethers is also possible and leads to degradation of drug substance.[3,6] Despite this known reactivity, there has generally been only scattered reporting of HPO levels in common pharmaceutical excipients.

Most of these investigations have focused on liquid phase excipients such as polysorbates and polyethylene glycols.[7–13] These measurements were performed using several different techniques: the ferrous oxidation-xylenol orange (FOX2) method, the coupled oxidation of NADPH method and the iodometric method. More recently, Huang et al.[14] described the liquid chromatographic-electrochemical determination of residual H_2O_2 in polyethylene glycol (PEG) 400, polyvinlypyrrolidone (PVP), and polysorbate. This work demonstrated significant and variable trace levels of H_2O_2 in these excipients. The drawback to this methodology is that it does not measure the ROOH content of the excipients. The goal of this work was to monitor "total" HPO content ($ROOH + H_2O_2$) with a simple, rapid, and sensitive methodology for common pharmaceutical excipients, both soluble and insoluble. The need for a rapid and sensitive method that can be used to measure total HPOs in excipients led to the selection of the FOX2 assay, a sensitive and relatively simple technique for measuring total HPOs.[15] The FOX2 assay has been used to evaluate oxidative damage in human and plant tissues, and in polysorbate 80 (PS80).[11,16–18] For comparative purposes, we have also used a second HPO assay, involving triphenylphosphine (TPP), which is also selective for both ROOH and H_2O_2.[19,20] The TPP assay proceeds via a different reaction mechanism than FOX2 and this attribute can be used to validate the results from the two methods.

We report total HPO levels in 10 common pharmaceutical excipients, including PVP, hydroxypropyl cellulose (HPC), PS80, PEG 400, medium chain glycerides (MCGs), poloxamer, lactose, sucrose, microcrystalline cellulose, and mannitol. Total HPO levels range over three orders of magnitude for these excipients; from <10 nmole HPO/g for microcrystalline cellulose, lactose, sucrose, and mannitol to over 10000 nmole HPO/g for certain lots of PVP. A more detailed study of HPO content of HPC, PVP, and PS80 is presented, with data on multiple lots and different excipient grades. These studies include an evaluation of the HPO content of the same excipients from different vendors. Finally, for excipients with the highest total HPO content, the individual contributions from H_2O_2 and ROOH are measured and the implications discussed.

EXPERIMENTAL

Materials

The following excipients were used as received from commercial vendors: povidone supplied by BASF Corporation (Mount Olive, NJ), International Specialty Products (ISP, Wayne, NJ), and Acros Organics (Morris Plains, NJ), PEG supplied by Dow Chemical Company (Nitro, WV), PS80 supplied by Croda, Inc. (Mill Hall, PA), NOF Corporation (Tokyo, Japan), and Acros Organics, HPC supplied by Hercules/Aqualon (Wilmington, DE), lactose supplied by Foremost Farms USA (Rothschild, WI), mannitol supplied by Roquette (Gurnee, IL), avicel supplied by FMC Corporation (Philadelphia, PA), sucrose supplied by Mallinckrodt (Phillipsburg, NJ), poloxamer supplied by BASF (Roxbury, NJ), and MCGs supplied by Sasol (Witten, Germany). All excipients are compendial grade unless otherwise noted. The specific lot numbers of the excipients are included in the data tables. Catalase from bovine liver, xylenol orange (sodium salt), and butylated hydroxytoluene (BHT) were obtained from Sigma (St. Louis, MO). Ferrous ammonium sulfate was purchased from Aldrich (Milwaukee, WI). TPP and triphenylphosphine oxide (TPO) were purchased from Acros Organics. Sulfuric acid, methanol, and water were obtained from Fisher Scientific (Philadelphia, PA). All other purchased chemicals were of analytical grade or higher.

Hydroperoxide Assay 1: FOX2 Methodology

The FOX2 assay has been used in total HPO measurements for a range of applications and the chemical principles of the assay have been well studied.[21] The assay is based on the reduction of the HPO by Fe(II) under acidic conditions. The resulting ferric ion forms a strong complex with

xylenol orange that is measured spectrophotometrically at 560 nm. The reaction stoichiometry is approximately 2 moles of Fe(III) formed from 1 mole of HPO, for either H_2O_2 or ROOH.[21] For this work, the FOX assay version II (FOX2) is selected because it permits more accurate quantitation of the HPOs in common pharmaceutical excipients without detailed knowledge of the type of HPO (ROOH or H_2O_2) or the structure of the R group on ROOH.

The FOX2 reagent is prepared according to the method described in previous work.[22] The final FOX2 color reagent (CR) contains 4 mM BHT, 0.10 mM xylenol orange, 0.25 mM ferrous ammonium sulfate, and 25 mM sulfuric acid in 10/90: water/methanol. The final blank reagent (BR) contains the same components as the CR except ferrous ammonium sulfate. The CR and BR are prepared fresh daily in sufficient volume such that all sample controls and the standard solutions can be prepared using the identical preparation of reagent solutions. UV/Vis analysis is performed using an HP 8453 UV/Vis Spectrophotometer from Agilent (Palo Alto, CA) with a 1 mL micro volume quartz cuvette. The absorbance of sample and standard solutions is measured at 560 nm with background subtraction at 900 nm.

Excipients samples are prepared between 0.02 and 0.001 g/mL of the color reagent which is within the linear response of the UV measurement. In most cases the excipient is added into a vigorously stirring solution in order to avoid gel formation. Multipoint stir plates are used to stir all samples at a fixed RPM. The solutions of insoluble excipients are clarified through a two-step process involving centrifugation and then filtration before UV analysis. The filtration procedure requires pretreating the syringes with the color reagent, as the syringes appear to have some HPOs present. A standard curve of H_2O_2 with several concentrations between 0 and 15 μM is prepared each day and the response is used to calculate the concentration of HPOs in excipients.

The sample testing methodology is optimized to increase the method precision. For each excipient tested, four sample solutions are prepared: in the color reagent, in the reagent blank, and two control solutions (CR and RB without excipient). All solutions are stirred at a controlled RPM for the same length of time (2 h) to ensure sample homogeneity and reproducible background oxidation of Fe(II). The control solution of color reagent is used to account for Fe(III) present in the ferrous ammonium sulfate and for the background oxidation of color reagent—Fe(III) formed by the slow oxidation of Fe(II). The reagent blank control is used to account for interfering species such as residual Fe(III) in glassware, manufacturing equipment, and excipients.

Hydroperoxide Assay 2: TPP/TPO Methodology

Triphenylphosphine is a convenient HPLC-based methodology for determining the H_2O_2 and ROOH content of solutions. The methodology has been published previously.[19] TPP reacts rapidly and quantitatively with H_2O_2 or ROOH to give 1 mole of TPO for each mole of HPO reacted.[20] TPP and TPO are easily resolved chromatographically. Excipients are added to a standard solution of 0.1 mg/mL TPP in 100% methanol. The sample is then allowed to react for 15 min prior to HPLC analysis. TPO area is compared to that from an identical sample without excipient added as a control. With a detection wavelength of 203 nm, the response factors of TPP and TPO are similar. The TPO peak area is converted to TPO concentration by using the TPP standard area and concentration. The TPP/TPO chromatographic conditions are as follows: column; 5 cm × 4.6 mm Synergi Polar-RP, column temperature 40°C, flow rate 1.0 mL/min, injection volume 10 μL, detection wavelength 203 nm, isocratic mobile phase of 75% methanol/25% water with a run time of 12 min. All HPLC analyses were performed on an Agilent 1100 (Agilent, Wilmington, DE) HPLC instrument with diode array detection.

Organic HPO and Hydrogen Peroxide Distribution: Catalase Methodology

Catalase is used to identify the type of HPOs present in PEG, HPC, PVP, and PS80. Catalase reacts with H_2O_2 but not with ROOH, and can be used to distinguish between these two HPOs in excipients. Excipient is reacted with catalase and then tested by the FOX2 assay for the remaining HPO concentration, which is only ROOH. The difference in FOX2 absorbance between excipient with catalase and excipient without catalase shows the amount of H_2O_2 in excipient. Spike and recovery experiments are conducted to demonstrate the activity of catalase in the presence of PS80.

A solution of catalase is prepared at 1200 activity units/mL in water. Concentrated excipient solutions are prepared in water at approximately

3–10× the concentration used in the FOX2 assay experiments. After mixing, the concentrated excipient solution is divided into two portions and mixed in a 5:1 ratio with catalase solution and with water, respectively. After 1 h of reaction, these two portions are each mixed with the FOX2 color reagent. All other testing methodology is as described for FOX2.

RESULTS

Overview of HPOs in Common Pharmaceutical Excipients

The HPO results for 10 common pharmaceutical excipients are presented in Table 1 with measurements performed using the FOX2 assay. Table 1 gives average HPO values as well as the highest and lowest HPO values determined for multiple lots tested. The excipients in Table 1 are listed in order from the highest average HPO determined (PVP, 7300 nmole/g) to the lowest values of <10 nmole/g for MCG, microcrystalline cellulose, mannitol, lactose, and sucrose. Overall PVP, PEG 400, PS80, and HPC contain significant levels of HPOs. In these cases, the difference in HPO content between different excipient lots and grades can be significant and is much larger than the measured RSD (<10%) for multiple preparations of the same lot. MCG and the common formulation diluents/fillers like mannitol and lactose contain low HPO levels. The HPO content of microcrystalline cellulose (avicel PH 101, 102, 105, and 200 grades) was also found to be less than 10 nmole/g.

A comparison of HPO values from the manufacturer certificate of analysis to the FOX2 method is shown in Table 2. It is evident that there is no standardized practice for measuring or reporting peroxide values for oxidizable excipients like PS80, PEG, and povidone. For example, not all grades of povidone or batches of PS80 are tested for peroxides even though peroxides are expected to be present. The certificate of analysis for HPC does not contain HPO-testing results (data not shown). Even when peroxide values are reported, these values are lower than when determined using the FOX2 (and TPP) method. These differences underscore the need for active monitoring of excipients by end users and also highlight the importance of using one assay methodology to test all excipients.

Table 3 shows total HPO determinations for specific lots of povidone, PEG 400, PS80, HPC, MCG, and avicel using both the FOX2 and the TPP/TPO methodologies. Overall the HPO results from both methodologies agree well in that the same ranking of high HPO and low HPO content is generally obtained across all the various excipients as well as within the multiple lots of each excipient tested. This shows there are no large differences in selectivity between the reactivity of the Fe (II) reagent and the reactivity of TPP. There are, however, some interesting excipient-specific trends in Table 3. The agreement between the HPO values measured by FOX2 and TPP/TPO assay is essentially quantitative for HPC and

Table 1. Measured Level of Hydroperoxides in a Set of Common Pharmaceutical Excipients

Excipient	# Lots Tested	Average HPO[a] (nmole/g)	High HPO Lot (nmole/g)	Low HPO Lot (nmole/g)
PVP	5	7300	11000	3600
PEG 400	4	2200	3300	1000
PS80	8	1500	4600	180
HPC	21	300	890	50
Poloxamer[b]	7	30	50	10
PEG solid[c]	4	20	40	<10
MCG	3	<10	<10	<10
Microcrystalline cellulose	5	<10	10	<10
Mannitol	5	<10	<10	<10
Lactose	5	<10	10	<10
Sucrose	5	<10	20	<10

[a]Hydroperoxide levels were acquired using the FOX2 assay. In some cases data reflect mulitiple vendors and grades of excipient. The overall trend shows that PVP, PEG 400, and PS80 have consistently higher HPO levels. HPC can also have significant levels. Poloxamer HPO content is low and the remaining excipients have very low HPO content at the detection limit of the FOX2 method.
[b]Different batches and grades (188, 338, and 407) of poloxamer solid are included.
[c]Testing is performed on batches of PEG 3400, 4600, and 6000.

Table 2. Comparison of HPO Content: FOX2 Assay and Excipient Certificate of Analysis

Excipient & Grade	Manufacturer and Lot Number	HPO/Peroxides from C of A	HPO (nmole/g)
PVP K17	BASF 20713588Q0	67 mg/kg	7800
PVP K29	ISP 05200087543	24 ppm	3900
PVP K29	ISP 05500129956	24 ppm	5200
PVP K90	ISP 03400121902	NR	7000
PVP K90	ISP 03500132524	NR	8800
PS80	Croda T4H-1033	0.00 mEq^02/kg	1100
PS80	Croda T4H-1034	0.00 mEq^02/kg	2100
PS80	Croda 0000114277	0.10 mEq^02/kg	750
PS80	Croda 0000136437	0.08 mEq^02/kg	570
PS80	NOF 402TA52	NR	1600
PS80	NOF 502TA51	NR	7700
PEG 400	Dow RD0755S4D2	NR	730
PEG 400	Dow QJ1155S4D5	NR	1100
PEG 4000	Dow RF1755S7B1	NR	<10
PEG 4600	Dow QK0555S7D1	NR	<10

NR, A peroxide # is not reported on the vendor certificate of analysis.
It is estimated that 1 ppm peroxide or mg peroxide/kg = 30 nmole HPO/g and 0.1 mEq^02/kg = 300 nmole HPO/g.

Table 3. Comparison of HPO Content: TPP/TPO Versus FOX2 Methodologies

		HPO (nmole/g)	
Excipient	ID of Lot & Grade	FOX2	TPP/TPO
PVP	K12, Acros A0180479[a]	2300	4300
	K17, BASF 20713588Q0	7800	15000
	K29, Acros A0189374[a]	3500	6700
	K29, ISP 05200087543	3900	7400
	K90, Acros A0159153[a]	13000	22000
	K90, ISP 03400122434	8900	15000
PEG 400	Dow RD0755S4D2	730	2500
	Dow QJ1155S4D5	1100	3300
	Dow QH2355S4D3	3200	5700
PEG 3350	Dow SB2655S7B1	<10	20
PEG 4000	Dow RF1755S7B1	<10	20
PEG 4600	Dow QK0555S7D1	<10	<10
PS 80	Croda T4H-1033	1100	1100
	Croda T4H-1014	1500	1400
	Sigma Aldrich 09414KO[a]	4600	4300
HPC	LF Grade Lot 9899	440	630
	LF Grade Lot 9159	450	390
	LF Grade Lot 4718	750	680
	EF Grade Lot 9897	560	760
Avicel	PH 101 FMC Lot 1321	<10	30
	PH 102 FMC Lot 2462	<10	20

Comparison of hydroperoxide assay results using FOX2 and TPP/TPO assays. In this case each excipient is sourced from the same vendor. The results are in good agreement in regard to overall HPO trends among the different excipients.
[a]Noncompendial grade.

PS80. However, for PVP and PEG 400, the TPP/TPO methodology gives total HPO values approximately two times larger than the FOX 2 assay. These excipient-specific trends will be discussed later.

Variable HPO According to Lot and Grade

Table 3 shows three- to sixfold variation in HPO content between different lots of the same excipient from the same vendor. Different HPO contents of different grades of the same material are also evident. This variation prompted a further investigation into the HPO content of several excipients. First, the total HPO content of various grades and lots of HPC was investigated, the resulting HPO data are shown in Table 4a,b. Table 4a shows 15 lots of LF grade HPC from the same vendor. The HPO content ranges from about 100 to 900 nmoles/g with no clear trend. Table 4b shows HPO data for different grades of HPC listed from the highest molecular weight material to the lowest. Once again there is no trend between the total HPO content and the average molecular weight of the HPC.

Polyvinlypyrrolidone and PS80 (Tabs. 1, 3) show high and variable HPO content and were also examined in more detail. Table 5 shows the PVP HPO content as a function of average molecular weight and vendor. From Table 5 there is a correlation between HPO content and

Table 4a. Detailed Study of Hydroperoxides in HPC (LF Grade)

Lot ID	HPO (nmole/g)	RSD (%)
3994	890	0.2
4362	440	4.0
4360	500	5.4
4718	750	1.3
5047	110	1.7
5825	140	6.3
6648	200	1.2
6832	210	3.2
9137	220	3.3
7622	270	3.9
9159	450	1.2
7616	220	11.2
8592	150	9.9
8604	100	17
8940	130	9.6

HPO Assay of 15 different lots of HPC LF grade material. Significant variation of HPO content is observed. All HPC are from same vendor.

the average molecular weight of the PVP; the larger the molecular weight the larger the amount of HPO/g. Table 5 also highlights that significant differences in HPO content (for similar average molecular weight material) between different vendors can be expected. Table 6 shows HPO data for PS80 from different vendors and batches. The HPO content in PS80 varied most significantly by manufacturer. As noted earlier, not all vendors report HPO levels for PS80. Interestingly, non-compendial PS80 from Acros contains low HPO levels comparable to many compendial batches.

Table 4b. Detailed Study of Hydroperoxides in HPC (Six Different Grades of HPC)

Grade	Average Molecular Weight	Lot #	HPO (nmole/g)	RSD (%)
HF	1150000	9821	330	14
GF	850000	9642	80	11
JF	370000	9686	50	1.5
LF A	140000	9843	150	2.0
LF B	95000	9899	440	0.6
EF	80000	9897	560	5.8

HPO Assay of six batches of HPC, one lot from grade of commercially available material. No clear trend between HPC molecular weight and HPO assay is evident.

Table 5. Study of Hydroperoxides in Different Grades and Batches of PVP

Grade	Manufacturer	Lot #	HPO (nmole/g)
K17	BASF	20713588Q0	7800
K17	BASF	34-0462	6900
K29	ISP	TX70720	10000
K29	ISP	05100056272	3600
K29	ISP	05200087543	3900
K29	ISP	05500129956	5200
K90	ISP	03400121902	7000
K90	ISP	03400122434	9000
K90	ISP	03500132524	8800

Manufacturer & Lot Number	Average Molecular Weight	HPO (nmole/g)
Acros Lot A0180479[a]	3500	2300
Acros Lot A0199571[a]	8000	2800
Acros Lot A0189374[a]	58000	3500
Acros Lot A0159153[a]	1300000	13000

Significant differences in HPO content is observed for different grade of PVP. The method RSD for HPO measurement of povidone is 1.7%.
[a]Noncompendial grade.

Hydrogen Peroxide and Organic HPOs in Excipients

The distribution of the total HPO content into ROOH and H_2O_2 in selected lots of PVP, PEG 400, PS80, and HPC is shown in Table 7 using the catalase methodology. Three of the four excipients (PEG 400, PVP, and HPC) contain various mixtures of ROOH and H_2O_2, while PS80 is shown to contain essentially all ROOH and no H_2O_2. Our catalase validation results confirm that the catalase was not inactivated by PS80 (data not shown). Similar research on polysorbate 20 and 80 has found significant H_2O_2 levels.[14,19] These results may not be comparable to our work because of differences in storage conditions (exposure to fluorescent light for 11 days) and possible chromatographic interference observed in the LC-wired enzyme testing methodology.

DISCUSSION

Formulating Oxidatively Sensitive Drug Substances

Solid Dosage Forms

Our findings are the first report of the total HPO levels using the same assay methodology in a broad range of common pharmaceutical excipients. It is clear that certain excipients like PVP,

Table 6. Variation of Hydroperoxides in Polysorbate 80

Manufacturer	Lot #	HPO (nmole/g)
Croda	T4h-1014	1500
Croda	T4h-1024	1000
Croda	T4h-1027	980
Croda	T4h-1028	710
Croda	T4h-1033	1100
Croda	T4h-1034	2100
Croda	0000114277	750
Croda	0000136437	570
NOF	402TA52	1600
NOF	502TA51	7700
ACROS	A017181501[a]	800
ACROS	A016198601[a]	290
Sigma-Aldrich	09414KO[a]	4600
Sigma-Aldrich	1312CA[a]	1600

Significant differences in HPO content are observed for different manufacturers of polysorbate 80. The method RSD for HPO measurement of polysorbate 80 is 2.3%.

[a]Noncompendial grade.

PEG 400, PS80, and HPC have significant HPO levels and that these levels may vary across different grades and between manufacturers of the same grade of excipient. In solid dosage forms, PVP is commonly used as a binder for wet granulation and is often used at fairly low levels. However, the total HPO content is high enough in PVP to promote significant degradation levels when formulating an oxidatively sensitive drug substance. Five percent PVP was shown to be responsible for N-oxide formation of raloxifene hydrochloride, due to the high HPO content.[6] Similarly, HPC is used at low levels in solid dosage forms and may introduce HPOs into the formulation. During a wet granulation process of a thio-ether with HPC, we observed sulfoxide formation which was related to the HPOs in the HPC.[23] HPO growth was also observed during wet granulation of the HPC containing placebo. The degradation of raloxifene hydrochloride and of the thio-ether are examples of the two-electron nucleophilic reaction that can occur between HPO and drug substance. Low-level HPO content may also be sufficient to participate in peroxy radical chain propagation (initiated by metal, heat, or light). The radicals can behave as initiators for radical chain processes leading to significant HPO and radical concentrations from a small amount of starting HPO. These radical processes may result in significant degradation of drug substance via single- and two-electron reactions. Fortunately microcrystalline cellulose, lactose, and mannitol commonly used in solid

Table 7. Distribution of Hydrogen Peroxide and Organic Hydroperoxides in PEG, PS80, PVP, and HPC

Excipient	ID of Lot	Distribution of Hydroperoxides		HPO (nmole/g)
		% ROOH	% H_2O_2	
PVP	K12 Acros Lot A0180479[a]	80	20	2300
	K17 Acros Lot A0199571[a]	40	60	2800
	K29 Acros Lot A0189374[a]	60	40	3500
	K29 ISP Lot 05200087543	70	30	3900
	K90 Acros Lot A0159153[a]	80	20	13000
	K90 ISP Lot 03400121902	80	20	7000
PEG 400	Dow RD0755S4D2	50	50	730
	Dow QJ1155S4D5	60	40	1100
	Dow QH2355S4D3	80	20	3200
PS 80	Croda T4H-1033	100	0	1100
	Croda T4H-1014	100	0	1500
	Croda T4H-1028[b]	100	0	3900
HPC LF	Hercules Lot 4360	30	70	500
	Hercules Lot 9899	40	60	440
	Hercules Lot 9159	50	50	450
	Hercules Lot 4718	30	70	750
	Hercules EF Lot 9897	80	20	560

[a]Noncompendial grade.

[b]Stored under ambient laboratory conditions for approximately 18 months.

dosage forms are relatively low in HPO content. Controlling HPO–drug reactions in solid dosage forms can be achieved by selecting excipients with low HPO levels, controlling crystallinity of the drug substance, and by adding antioxidants to stop HPO propagation and single-electron degradation processes.

Lipid-Based Oral Formulations

Achieving adequate exposure of the drug substance can be a challenge especially for poorly water-soluble compounds. This goal has driven use of lipid-based dosage forms, in which drug substances are formulated with glycerides or surfactants such as MCG, PEG 400, poloxamer, and PS80.[24,25] The HPO data in Tables 1–4 clearly show that PS80 and PEG 400 have much higher HPO levels than MCG and poloxamer. Liquid formulations using PS80 and PEG 400 represent a highly "oxidizing" environment that can be conducive to radical chain degradation reactions as well as the two-electron nucleophilic reactions. The reactivity of drug substance with HPO is further enhanced by the amorphous nature of drug substance and excipients in these types of formulations. Thus, every effort should be made to understand the reactivity of the drug substance toward both HPOs (N-oxide, sulfoxide type reactivity) as well as toward peroxy radicals.[26] If a drug substance is shown to be sensitive to these types of oxidation reactions, PS80 and PEG 400 should be avoided and surfactants such as MCG and poloxamer should be evaluated. The addition of antioxidant may also be necessary for drug substances that are sensitive to oxidation.

Relationship between HPOs and Excipient Manufacturing

An important finding of this article is that the HPO content of excipients may contain significant contributions from both ROOH and H_2O_2. These findings also introduce the possibility that different HPOs may yield different degradation kinetics. Although it may not be necessary to distinguish between ROOH and H_2O_2 in all cases, the potential different reactivities of different HPOs may have to be considered in specific cases of drug substance or product sensitive to oxidation.

A final aspect of the HPO distributions is to generally rationalize the data shown in Table 7 with respect to each excipient's known manufacturing process. In the case of PS80, there is no hydrogen peroxide used in the manufacturing process. PS80 itself has low energy C–H bonds which can react with peroxy radicals via hydrogen atom abstraction. Addition of molecular oxygen can then lead to a HPO group on the PS80 (an organic HPO). The oleic acid raw material also carries HPO groups. In Table 7, the HPO content for PS80 is exclusively ROOH with no appreciable H_2O_2. Similarly, the PEG 400 manufacturing process does not use hydrogen peroxide, and PEG 400 also has oxidizable C–H bonds. Thus, we would anticipate PEG 400 would be dominated by ROOH. Table 6 shows that 20–50% of the HPO content in PEG 400 is actually due to hydrogen peroxide. We can only speculate that after manufacture, PEG 400 slowly oxidizes and some HPO groups are eliminated as hydrogen peroxide. It is also possible that the starting materials contain low-level hydrogen peroxide impurities from their respective syntheses or that some type of bleaching with H_2O_2 has been utilized.

Polyvinlypyrrolidone is synthesized from N-vinylpyrolidone via a free-radical polymerization reaction from which oxygen is often excluded as much as possible.[27] However, trace levels of oxygen remain that react with the polymerizing free radicals to form HPO on the PVP backbone. Thus a clear rationale for ROOH in PVP exists. Hydrogen peroxide is also used at elevated temperatures to initiate the free-radical polymerization and may be present as an impurity from the manufacturing process. Table 7 shows that PVP HPO content is typically dominated by ROOH, but can have between 20% and 60% hydrogen peroxide.

Finally, HPC is a slightly different case in which the base cellulose is extracted and purified from natural sources. The cellulose ether is then reacted with propylene oxide under elevated temperature and pressure. Hydrogen peroxide or tert-butyl hydroperoxide treatment is commonly used to reduce the average molecular weight of the cellulose and residual levels may remain in HPC as a processing impurity.[28–30] However, no trend of HPO content with molecular weight was noted in Table 4a,b. Increasing degrees of cellulose chain cleavage do not lead to systematically higher HPO levels. It is possible that the starting cellulose material carries some amount of intrinsic HPO content. The oxidation of low energy C–H bonds on HPC or on impurities may also be responsible for some of the HPO content.

Attributes of HPO Testing Methodology

Although most of our HPO characterization has been performed using the FOX2 method, a comparison of the key method attributes shows that either FOX2 or TPP/TPO may be used for HPO testing of excipients. One key similarity is that both methods are sensitive to the two classes of HPO: hydrogen peroxide and organic HPOs. In addition, each method can be used to generate rapid and sensitive HPO measurements with a precision (typical RSD $< 3\%$) that is low enough for discriminating comparisons of different batches of excipients. These methods also yield HPO results showing good agreement for excipients like PS80 and HPC. However, as shown in Table 3 the HPO results for PVP and PEG were significantly higher when using the TPP/TPO method. For all samples, this difference appears to be reproducible, systematic, and excipient specific and was therefore investigated. The possibility of differential reactivity of FOX2 and TPP reagents with peroxides (ROOR) and HPOs was explored. Based on our own findings, the difference is not related to incomplete reactivity with hydrogen peroxide or organic HPOs. In addition, the reactivity of FOX2 and TPP with peroxides does not explain the trends for PVP and PEG since our research indicates neither method is reactive with simple alkyl peroxides and other investigators have shown that FOX2 is more reactive with cyclic peroxides.[31] One explanation for this difference is that non-HPO impurities or functional groups specific to PVP and PEG are responsible for the different HPO results. It is important to note that these non-HPO impurities appear related to the HPO levels in the excipient. For example, the HPO content of PVP tested by TPP/TPO and FOX2 is different by a factor of approximately $1.8\times$ for all batches and grades even spanning a $5\times$ range of HPO values. This off-target reactivity leads to the different and yet reproducible HPO results observed for PVP and PEG by either of two scenarios: reaction with TPP to yield inflated HPO results compared to FOX2 or by reduction of the FOX2 signal (xylenol orange–Fe(III) complex) leading to depressed HPO results compared to the TPP result. It is difficult to identify the exact cause without detailed knowledge of the full excipient matrix for PVP and PEG as well as the exact structure of the organic HPOs which appear related to this off-target reactivity/matrix effect. Hence, an important objective for future work will be characterization of the off-target reactivity/excipient matrix for PVP and PEG as well as structural information on the HPOs that appear to influence this effect. Despite the differences in Table 3, the HPO values obtained by FOX2 and TPP/TPO have the same general trends among all excipients and among different grades of the same excipient (ex. PVP). Either method can be used to provide similar information on common pharmaceutical excipients with the exception that the same HPO assay should be used when running comparative studies of excipients that include PEG and PVP.

Acceptable HPO Limits Are Related to Chemistry of Drug Substance or Drug Product

Our findings show that common excipients like PVP, PEG 400, PS80, and HPC contain significant HPO levels even though these are compendial grade. In addition our results indicate considerable variability according to grade, batch, and vendor and different practices for peroxide monitoring by manufacturers. Some of the variability may be related to differences in manufacturing process, impurities in raw materials, bleaching or storage conditions. These differences in addition to HPO levels may not be communicated to end users by the manufactures in part because it is difficult to assign general limits on HPO levels in excipients. These limits can only be properly assigned in the context of a particular formulation and active pharmaceutical ingredient. Thus, when formulating oxidatively sensitive drug substances (especially those in an amorphous state), carefully monitoring HPO content falls to the excipient user.

Even when oxidation of drug substance is not a problem, the HPO levels in excipients may lead to other formulation challenges. Other researchers have shown that HPO levels and oxidizability of excipients can lead to significant changes in pH, appearance, and viscosity.[32–34] Furthermore, the decomposition of HPO may yield unwanted impurities such as aldehydes and carboxylic acids. Aldehydes are also known to promote undesirable capsule cross-linking in both hard and soft gelatin capsules.[35] Once again acceptable HPO limits can only be assigned within the context of a particular formulation that is known to be sensitive to the HPOs and oxidation. In this case, the acceptable limits for HPO content could be defined by understanding the relationship between HPOs and formulation quality. We hope that our findings

emphasize that excipient equivalence can not be assumed when considering HPO content and that monitoring HPO content of excipients may be prudent in specific cases defined by the chemistry of the drug substance or drug product.

CONCLUSION

The role of HPOs in the oxidative degradation of a drug substance necessitates monitoring the HPO content of excipients and formulated product. In this study, the FOX2 and TPP/TPO assays were used to produce quick, accurate, and sensitive measurements of HPO concentrations for 10 common pharmaceutical excipients. Some excipients like HPC, PEG, PVP, and PS80 were found to contain significant HPO levels. Other excipients such as lactose, sucrose, avicel, and mannitol have very low HPO levels. For those excipients with significant HPO content, considerable variation in HPO content across different batches and vendors may occur. For these excipients, active monitoring and control of HPOs by the suppliers may be necessary. The application of the HPO measurement described in this article is not limited to the testing of starting excipients but may also be performed on the formulated drug product when needed. The HPO assays could be used in comparative studies of HPO levels. These studies could involve different batches and types of excipients and may lead to the selection of excipients and manufacturing processes that minimize HPO concentration and enhance drug product stability. This methodology would be particularly useful for drug substances prone to HPO-mediated degradation pathways and could be an effective way to avoid, solve, or minimize unexpected stability problems.

REFERENCES

1. Landin M, Martinez-Pacheco R, Gomez-Maoza JL, Suoto C, Concheiro A, Rowe RC. 1993. Effect of batch variation and source of pulp on the properties of microcrystalline cellulose. Int J Pharm 91:133–141.
2. Shah U, Augsburger L. 2001. Evaluation of the functional equivalence of crospovidone NF from different sources. I. Physical characterization. Pharm Dev Tech 6:39–51.
3. Hovoroka SW, Schoneich C. 2001. Oxidative degradation of pharmaceuticals: Theory, mechanisms and inhibition. J Pharm Sci 90:253–268.
4. Johnson DM, Gu LC. 1988. Autoxidation and antioxidants. In: Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology, Vol. 1. New York: Wiley. pp 415–449.
5. Sheldon RA, Kochi JK. 1976. Metal-catalyzed oxidations of organic compounds in the liquid phase: A mechanistic approach. In: Eley DD, editor. Advances in catalysis. New York: Academic Press. pp 273–413.
6. Hartauer KJ, Arbuthnot GN, Baertschi SW, Johnson RA, Luke WD, Person NG, Rickard EC, Tingle CA, Tsang PKS, Wiens RE. 2000. Influence of peroxide impurities in povidone and crospovidone on the stability of raloxifene hydrochloride in tablets: Identification and control of an oxidative degradation product. Pharm Dev Tech 5:303–310.
7. Donbrow M, Azaz E, Pillersdorf A. 1978. Autoxidation of polysorbates. J Pharm Sci 67:1676–1681.
8. Chang HW, Bock E. 1980. Pitfalls in the use of commercial nonionic detergents for the solubilization of integral membrane proteins: Sulfydryl oxidizing contaminants and their elimination. Anal Biochem 104:112–117.
9. Knepp VM, Whatley JL, Muchnik A, Calderwood TS. 1996. Identification of antioxidants for prevention of peroxide-mediated oxidation of recombinant human ciliary neurotropic factor and recombinant human nerve factor. J Pharm Sci Technol 50:163–171.
10. Mancuso JR, McClements DJ, Decker EA. 1999. Ability of iron to promote surfactant peroxide decomposition and oxidize α-tocopherol. J Agric Food Chem 47:4146–4149.
11. Ha E, Wang W, Wang J. 2002. Peroxide formation in polysorbate 80 and protein stability. J Pharm Sci 91:2252–2264.
12. McGinity JW, Patel TR, Naqvi AH. 1976. Implications of peroxide formation in lotion and ointment dosage forms containing polyethylene glycols. Drug Dev Comm 2:505–519.
13. Bindra DS, Williams TD, Stella VJ. 1994. Degradation of O6-benzylguanine in aqueous polyethylene glycol 400 (PEG 400) solutions: Concerns with formaldehyde in PEG 400. Pharm Res 11:1060–1064.
14. Huang T, Garceau ME, Gao P. 2003. Liquid chromatographic determination of residual hydrogen peroxide in pharmaceutical excipients using platinum and wired enzyme electrodes. J Pharm Bio Anal 31:1203–1210.
15. Jiang ZY, Hunt JV, Wolff SP. 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in low density lipoprotein. Anal Biochem 202:384–389.
16. Gay C, Collins J, Gebicki JM. 2003. Measurement of protein and lipid hydroperoxides in biological systems by the ferric-xylenol orange method. Anal Biochem 315:29–35.

17. DeLong JM, Prange RK, Hodges DM, Forney CF, Bishop MC, Quillam M. 2002. Using a modified ferrous oxidation-xylenol orange (FOX) assay for detection of lipid hydroperoxides in plant tissue. J Agric Food Chem 50:248–254.
18. Jaeger J, Sorensen K, Wolff SP. 1994. Peroxide accumulation in detergents. J Biochem Biophys Methods 29:77–81.
19. Nakamura T, Maeda H. 1991. A simple assay for lipid hydroperoxides based on triphenylphosphine oxidation and high-performance liquid chromatography. Lipids 26:765–768.
20. Rowley AG. 1979. Conversions via oxygenation. In: Cadogan JIG, editor. Organophosphorous reagents in organic synthesis. New York: Academic Press. pp 318–327.
21. Gay C, Collins J, Gebicki JM. 1999. Hydroperoxide assay with the ferric-xylenol orange complex. Anal Biochem 273:149–155.
22. Nourooz-Zadeh J, Tajaddini-Sarmadi J, Wolff SP. 1994. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. Anal Biochem 220:403–409.
23. Harmon PA, Wuefling WP, Harman AB, Wasylaschuk WR, Givand J, Shelukar S, Reed RA. 2004. Role of organic hydroperoxides on process robustness and finished product quality (presented at AAPS).
24. Humberstone AJ, Charman WN. 1997. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Del Rev 25:103–128.
25. Poton CW. 2000. Lipid formulations for oral administration of drugs: Non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. Eur J Pharm Sci 11:S93–S98.
26. Harmon PA, Kosuda K, Nelson E, Mowery M, Reed RA. 2006. A novel peroxy radical based oxidative stressing system for ranking the oxidizability of drug substance. J Pharm Sci (in press).
27. Buhler V. 1999. Kollidon: Polyvinylpyrrolidone for the pharmaceutical industry. BASF 9–12.
28. Kroschwitz JI, Howe-Grant M, editors. 1993. Cellulose ethers. In: Kirk-Othmer encyclopedia of chemical technology, Vol. 5. New York: John Wiley & Sons. pp 541–542.
29. U.S. Patent. 2,512,338 (June 20, 1950), E.D. Klug and H.M. Spurlin (to Hercules Powder Co.).
30. U.S. Patent 3,719,663 (March 6, 1973), E.D. Klug (to Hercules Inc.).
31. Yin H, Porter NA. 2003. Specificity of the FOX assay: Analysis of autoxidation products of cholesteryl arachidonate by the TBARS and FOX assays. Anal Biochem 313:319–326.
32. Johnson DM, Taylor WF. 1984. Degradation of fenprostalene in polyethylene glycol 400 solution. J Pharm Sci 84:1414–1417.
33. Kasraian K, Kuzniar AA, Wilson GG, Wood JA. 1999. Developing an injectable formula containing an oxygen-sensitive drug: A case study of danofloxacin injectable. Pharm Dev Tech 4:475–480.
34. Dahl T, He GX, Samules G. 1998. Effect of hydrogen peroxide on the viscosity of a hydroxyethylcellulose-based gel. Pharm Res 15:1137–1140.
35. Digenis GA, Gold TB, Shah VP. 1994. Cross-linking of gelatin capsules and its relevance to their in vitro-in vivo performance. J Pharm Sci 83:915– 921.