Hydrolysis of the Quinone Methide of Butylated Hydroxytoluene in Aqueous Solutions

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ABSTRACT: Butylated hydroxytoluene or BHT is an antioxidant commonly used in pharmaceutical formulations. BHT upon oxidation forms a quinone methide (QM). QM is a highly reactive electrophilic species that can undergo nucleophilic addition. Here, the kinetic reactivity of QM with water at various apparent pH values in a 50% (v/v) water–acetonitrile solution at constant ionic strength of $I = 0.5$ $(NaCl)_4$, was studied. The hydrolysis of QM in the presence of added acid, base, sodium chloride, and phosphate buffer resulted in the formation of only one product—the corresponding 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol (BA). The rate of BA formation was catalyzed by the addition of acid and base, but not chloride and phosphate species. Nucleophilic excipients, used in the pharmaceutical formulation, or nucleophilic groups on active pharmaceutical ingredient molecule may form adducts with QM, the immediate oxidative product of BHT degradation, thus having implications for drug product impurity profiles. Because of these considerations, BHT should be used with caution in formulations containing drugs or excipients capable of acting as nucleophiles. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: hydrolysis; butylated hydroxytoluene; BHT; antioxidants; quinone methide; adduct; chemical stability; degradation products; kinetics

INTRODUCTION

Butylated hydroxytoluene (BHT) is used as an antioxidant in many products, including food, pharmaceuticals, cosmetics, jet fuels, rubber, paint, and petroleum products. It is also on the US Food and Drug Administration's (FDA) list of compounds generally recognized as safe (GRAS).[1] While there was some concern with the safety of BHT based on animal experiments,[2–9] the European Union recently increased the acceptable daily intake or ADI of BHT from 0.05 mg/kg per day to 0.25 mg/kg per day.[10]

The mechanism of the action of BHT as an antioxidant is through the formation of a stable phenoxyl radical, which further disproportionates to give the parent and a quinone methide (QM) [11] Scheme 1). This QM can then undergo further reactions with nucleophiles to form an adduct.

Ortho- and *para*-QMs are reactive electrophilic species and can be thought of as highly polarized, charge-separated species as depicted in Scheme 2.[12] This charge distribution characterizes the methylene carbon as the center of probable nucleophilic attack.

The reactivity of QMs is further enhanced by the aromatization of the phenyl ring in the product as a result of nucleophilic addition. This provides a strong thermodynamic driving force for addition of nucleophiles to the methylene group.

Quinone methides can easily form adducts with nucleophiles or polymerize. In pharmaceutical formulations, the possibility of adduct formation between the QM from BHT oxidation and a nucleophilic group of the active pharmaceutical ingredient (API) or another excipient is possible. To date, there is one report of API–QM adduct formation in a topical formulation.[13]

This research was undertaken to investigate the reactivity of QM with water under various pH conditions and with two possible nucleophilic species capable of adding to QMs, chloride ions, and various phosphate species around neutral pH. The focus of the work was to characterize the kinetics of the hydrolysis reaction. It is only with the understanding of the hydrolysis reaction that further characterization of the kinetics of reactivity of QM with additional nucleophiles can be understood.

MATERIALS AND METHODS

Materials

Butylated hydroxytoluene, lead dioxide, and 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol (BA) were purchased from Sigma–Aldrich (St. Louis, Missouri) and used without additional purification. All chemicals were of reagent grade. Sodium chloride, and disodium hydrogen phosphate were from JT Baker (Phillipsburg, New Jersey), sodium dihydrogen phosphate hepta-hydrate was from Mallinckrodt (Phillipsburg, New Jersey), and sodium perchlorate was from Acros (Geel, Belgium). Pentane (EMD Millipore, Billerica, MA), methanol (JT Baker), and acetonitrile (Sigma–Aldrich) were of high-performance liquid chromatography (HPLC) grade, purchased through VWR and Sigma–Aldrich, and were used for all sample preparations and analyses. Water for kinetics studies and HPLC analysis

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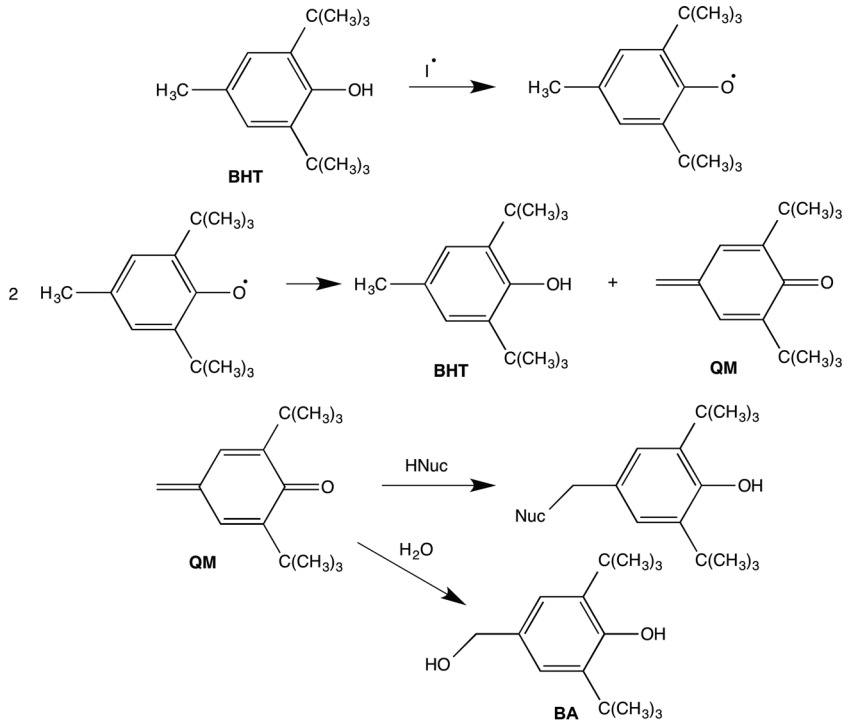
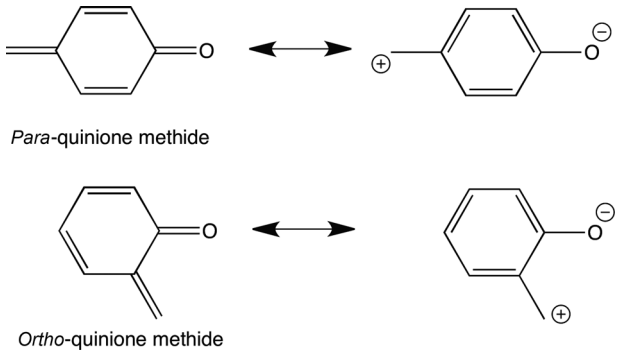


Scheme 1. Reaction pathways for the oxidation of BHT to give it its corresponding QM and the subsequent addition of a nucleophile (BHT adduct), including water for the corresponding BA.



Scheme 2. Resonance structures of *para*- and *ortho*-QMs.

was distilled and passed through a Nanopure water purification system from Thermo Scientific (Waltham, MA).

Generation of QM

Quinone methide was generated by modification of a published procedure.[14] A 0.001-M solution of BHT in pentane containing approximately 25–28 molar equivalents of PbO_2 was stirred at room temperature for 2 h. Following filtration through a 0.2-μm nylon syringe filter, an equal volume of acetonitrile was added and the mixture was rotary evaporated for 1 –1½ h at 230 mbar and 40°C to approximately 35% original volume. The resulting concentrate was added to a volumetric flask, and additional acetonitrile was added until the total volume was 10 mL. The yield of QM was approximately 30%. The solution was kept in the freezer for up to 1 week and further diluted for kinetics studies. Unreacted BHT did not appear to interfere with the kinetic analysis.

Determination of QM Concentration

The concentration of QM was determined using total ultraviolet (UV) absorbance at 285 nm and extinction coefficient values of $\varepsilon_{QM} = 2.82 \times 10^4\ cm^{-1}\ M^{-1}$ [11] and $\varepsilon_{BHT} = 2.16 \times 10^3\ cm^{-1}\ M^{-1}$ and Eqs. 1 and 2, (Ref. 15).

$$Abs_{total} = \varepsilon_{QM} C_{QM} + \varepsilon_{BHT} C_{BHT} \quad (1)$$

$$C_{total} = C^0_{BHT} = C_{BHT} + C_{QM} \quad (2)$$

where Abs_{total} is the observed absorbance reading, and the C values reference to molar concentrations.

Preparation of Buffer Solutions

Buffer stock solutions were made at 0.2 M and $I = 1.0$ ($NaClO_4$). Phosphate buffer stock was made by weighing out sodium dihydrogen phosphate, disodium hydrogen phosphate, and sodium perchlorate in appropriate amounts estimated to provide the desired pH value into a 100-mL volumetric flask and dissolving in approximately 80 mL of water. The pH was adjusted with sodium hydroxide and/or perchloric acid to the target pH value. Lower concentration stock solutions were made by diluting the 0.2-M stock with 1.0-M sodium perchlorate to maintain ionic strength and pH adjusted as above, if necessary. Buffers were stored refrigerated for up to 1 month.

pH Measurements

The pH measurements were performed at ambient conditions using a Thermo Orion 9863BN Needle Tip combination electrode from Thermo Orion (Waltham, MA). The electrode

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was calibrated with three standards, pH 4, 7, and 10, before each series of measurements.

HPLC Analysis

An Agilent 1100 and 1200 Series (Palo Alto, California) HPLC–UV spectroscopy instruments, each equipped with a binary pump (G1312A), DAD detector (G1315B), auto sampler (G1329A), and 15×4.6 mm^2 5 μm Phenomenex Luna C18 column (Torrance, CA) were used for the analysis. Elution was performed using a solvent gradient of 40%–100% B (B = 100% methanol) in solvent A (A = 40% methanol in water) for 20 min at a flow rate of 1 mL/min. Peaks were detected at 285 nm and Dionex Chromeleon analysis software (Sunnyvale, California) was used to integrate the peak area.

UV–Vis Spectrophotometry Analysis

Shimadzu UV-1700 (Nakagyo-ku, Kyoto, Japan) UV–Vis spectrophotometer equipped with deuterium and halogen lamps was used for sample analysis. For experiments in which the entire spectra were recorded, the spectra were zeroed at 400 nm and scanned from 400 to 190 nm with absorbance values calculated at 285 nm.

Kinetic experiments were followed at 285 nm with sampling time depending on the rate of the kinetic reaction. The data were transferred into excel spreadsheets for analysis. To account for potential drift in the baseline for the long duration runs, an empty cuvette was used as a reference at each time point. Drift was less than 0.004 of the total absorbance units throughout the experiments, so no correction was used.

Kinetic Studies

Kinetic studies were carried out at 25°C in a 50% (v/v) aqueous acetonitrile and constant ionic strength, $I = 0.5$ ($NaClO_4$). Reactions were initiated by mixing equal volumes of aqueous buffer solution and the acetonitrile solution of QM. The final concentration of QM in the reaction mixtures was $(1.75 - 4.71) \times 10^{-5}$ M, and the final buffer concentration varied from 0.005 to 0.05 M. After initiation, the pH_{app} was measured, and aliquots of the reaction mixture were transferred to HPLC vials and incubated in the thermostated HPLC sample holder at 25°C. In those studies, where UV–Vis spectrophotometry was also used, an additional aliquot was simultaneously transferred to a 1-cm path length quarts cuvette for UV–Vis analysis. For reactions too rapid for HPLC, UV–Vis analysis was used exclusively to monitor the reaction. The reactions were monitored at $\lambda = 285$ nm for both the loss of parent and the product formation by HPLC and for total absorbance by UV–Vis.

Generation of Standard Curves

A standard curve for QM was generated by diluting a preparation of QM with acetonitrile 10–100-fold and measuring total UV absorbance and HPLC area. The total absorbance was used to calculate the amount of QM at each concentration, and this was correlated with the HPLC area to produce a linear standard curve. Chromatograms showed only the presence of QM and BHT, and no degradation of QM was observed in the duration of the experiment.

The standard curves for BHT and BA were generated by weighing out different amount of solid standards and dissolving them in acetonitrile for UV–Vis and HPLC analyses. The lowest concentrations of BA were prepared by serial dilution, and in duplicate. Concentrations were corrected for the purity listed on the label and volatiles determined by thermogravimetric analysis.

Calculation of Rate Constants

Observed first-order rate constants were calculated as the slopes of the semilogarithmic plots of the HPLC area or change in UV absorbance (absorbance minus absorbance at infinity) against time. Observed second-order rate constants were calculated as the slopes of linear plots of the observed first-order rate constants versus nucleophile concentration. Rate constants for hydrolysis of QM were determined using linear regression analysis using Excel or GraphPad/Prism version 6.0 (GraphPad Software, La Jolla, California). Nonlinear regression analysis to generate the pH_{app}-rate profile used GraphPad/Prism version 6.0.

RESULTS

Hydrolysis of QM in 50% Water–Acetonitrile

The hydrolysis of QM was monitored by both HPLC and UV–Vis analysis in 50% (v/v) aqueous acetonitrile solutions at a constant ionic strength of $I = 0.5$ ($NaClO_4$) at 25°C. The disappearance of QM was accompanied by the appearance of a single peak corresponding to BA, by comparison of its HPLC retention time and measured UV–Vis spectrum with that of a commercial standard. A typical chromatogram for this reaction is show in Figure 1. It clearly indicates that QM, the residual BHT (see *Materials and Methods* section on this point), and BA can all be reasonably separated from one another. The level of BHT contamination in the QM solution remains unchanged throughout the course of the hydrolysis reaction.

Semilogarithmic plot of the loss of the QM versus time was linear, indicating that pseudo-first-order kinetics was followed. The observed first-order rate constant for the disappearance of the QM was $k_{obs} = 5.33\ (\pm 0.28) \times 10^{-2}\ h^{-1}$ (average of five runs shown in Table 1). The measured apparent pH (pH_{app}) of the solutions at the end of the reactions was 7.18 ± 0.10.

Hydrolysis of QM to BA in the Presence of Added Acid

The rate of formation of BA because of water addition to QM in acidic solutions is rapid and was followed by UV spectroscopy as HPLC analysis was too slow to capture the reaction kinetics. The reactions were carried out in solutions containing 0.001 – 0.02 M hydrochloric or perchloric acid in 50% (v/v) aqueous acetonitrile with a constant ionic strength of $I = 0.5$ ($NaClO_4$). Table 1 also summarizes the observed first-order rate constant values, k_{obs}, at various acid concentrations. At all concentrations of added acid, the reaction followed pseudo-first-order kinetics, and observed rate constants were calculated from plotting the logarithm of the change in UV absorbance at 285 nm versus time.

Figure 2 shows the dependence of the observed first-order rate constants for the acid-catalyzed hydrolysis on the concentration of acid, and no dependence on counter ion (chloride vs. perchlorate). The observed second-order rate constant for the acid-catalyzed addition of water to QM, k_H, was calculated as the slope of this plot, $k_H = 2.88\ (\pm 0.06) \times 10^3\ M^{-1}\ h^{-1}$.

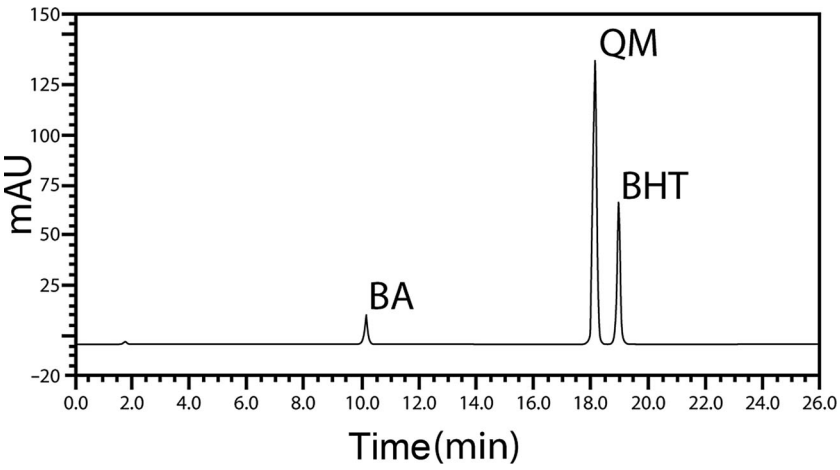


Figure 1. A sample HPLC chromatogram of the reaction of the QM of BHT with water in 50% (v) acetonitrile $I = 0.5$ ($NaClO_4$) without pH adjustment and the formation of BA at 25°C.

Hydrolysis of QM to BA in the Presence of Added Sodium Hydroxide

The hydrolysis of QM in basic pH solutions with added sodium hydroxide (0.005 –0.02 M) 50% (v/v) aqueous acetonitrile, with a constant ionic strength of $I = 0.5$ ($NaClO_4$), is rapid and was also followed by UV spectroscopy because of the speed of the reaction. Table 1 contains the observed first-order rate constant values, k_{obs}, at various hydroxide concentrations.

Figure 2 also shows the dependence of the observed first-order rate constants on the concentration of added hydroxide confirming the strong dependence of the loss of QM on hydroxide concentration. The observed second-order rate constant for the base-catalyzed addition of water to QM, k_{OH}, was calculated as the slope of this plot, $k_{OH} = 6.42\ (\pm 0.16) \times 10^2\ M^{-1}\ h^{-1}$.

Hydrolysis of QM in the Presence of Chloride Ion

The reaction of QM in the presence of the added chloride ion was examined at three concentrations of sodium chloride ranging from 0.05 to 0.1 M and monitored by HPLC analysis. Reactions

Table 1. Observed First-Order Rate Constant Values, k_{obs}, and pH_{app} Values for Hydrolysis of the QM of BHT in Unbuffered 50% (v/v) Aqueous Acetonitrile $I = 0.5$ ($NaClO_4$), and with Various Added Acids, HCl or $HClO_4$, Sodium Hydroxide, and Chloride and Phosphate Concentrations. Reaction kinetics were followed by UV–Vis at 25°C for the acid and base studies and by HPLC for the unbuffered, and added chloride and phosphate studies

QM (M)	4.71×10^{-5}	2.56×10^{-5}	2.93×10^{-5}	2.78×10^{-5}	1.75×10^{-5}			
k_{obs} (h^{-1})	5.37×10^{-2}	5.36×10^{-2}	5.08×10^{-2}	5.08×10^{-2}	5.75×10^{-2}			
pH_{app}	7.35	7.09	7.16	7.11	7.20			
$[H^+]$ (M)	0.001	0.005		0.0075	0.010		0.015	0.020
Acid	$HClO_4$	$HClO_4$	HCl	$HClO_4$	$HClO_4$	HCl	HCl	HCl
k_{obs} (h^{-1})	2.35	13.2	13.2	20.1	27.2	26.1	42.1	56.8
pH_{app}	2.81	2.06	2.30	1.83	1.71	1.94	1.74	1.63
$[OH^-]$ (M)	–	0.005	–	0.010	0.015	0.020		
k_{obs} (hr^{-1})	–	3.00	–	6.4	9.7	12.6		
pH_{app}	–	12.14	–	12.41	12.52	12.61		
$[Cl^-]$ (M)	0.050	0.075	0.100					
pH_{app}	7.2	7.5	7.8					
k_{obs} (h^{-1})	4.73×10^{-2}	4.84×10^{-2}	4.82×10^{-2}					
[Phos] (M)	0.005	0.025	0.050					
pH_{app}	7.40	7.28	7.20					
k_{obs} (h^{-1})	5.37×10^{-2}	5.18×10^{-2}	4.83×10^{-2}					

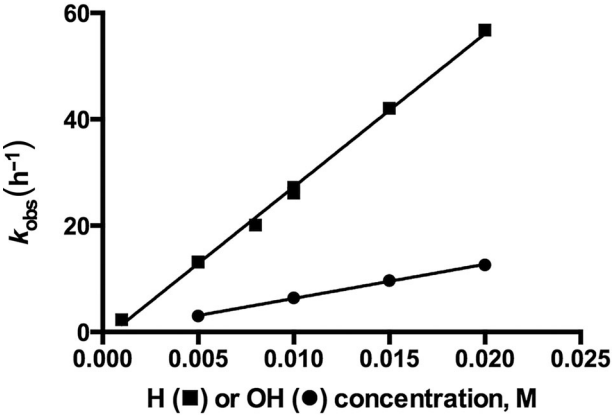


Figure 2. Dependence of the observed first-order rate constants for the hydrolysis of the QM of BHT on concentration of added acid, hydrochloric acid, and perchloric acid (■, check Table 1 for the conditions studied), and sodium hydroxide (●) in 50% (v/v) aqueous acetonitrile $I = 0.5$ ($NaClO_4$) at 25°C.

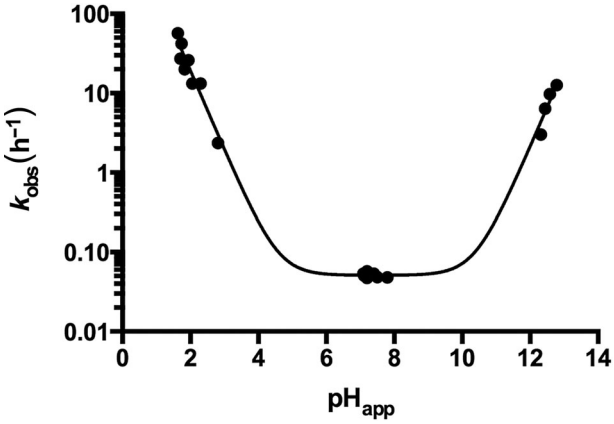


Figure 3. pH_{app}-rate profile for the hydrolysis of the QM of BHT to its corresponding BA in 50% (v/v) aqueous acetonitrile I = 0.5 ($NaClO_4$) at 25°C.

were first order with respect to the loss of QM, and the observed pseudo-first-order rate constants are also shown in Table 1.

Chromatograms for the reaction in the presence of added sodium chloride showed no additional product peaks besides the water addition product, BA, and approximate mass balance was observed throughout the course of the reaction.

Hydrolysis of QM in the Presence of Phosphate Buffer Species

The reaction of QM in the presence of phosphate buffer was examined at three concentrations ranging from 0.005 to 0.05 M and monitored by HPLC analysis. At the apparent pH values studied, the buffer species present are mono- and di-basic phosphate. Reactions were first order with respect to the loss of QM, and the observed pseudo-first-order rate constants are summarized in Table 1.

Chromatograms for the loss of QM in the presence of phosphate showed no additional product peaks besides the water addition product, BA, throughout the course of the reaction including any obvious perturbations at the solvent front. Extrapolation to $t = 0$ using the first-order rate constant revealed that the calculated initial QM concentration from the intercept was the same as the prepared initial concentration, within the error of the measurement, and approximate mass balance of QM to BA was observed over the course of the reaction. The solubility of phosphate buffer in 50% (v/v) aqueous acetonitrile in the pH range of interest prohibited testing the reactivity at higher concentrations or higher pH values of phosphate buffer.

pH_{app}-Rate Profile for the Hydrolysis of QM to BA

Figure 3 shows the overall dependency of all the observed first-order rate constants presented in Table 1 on pH. As the pH measurements were made in the 50% (v/v) acetonitrile water solvent, the values are reported as apparent pH values or pH_{app}.

DISCUSSION

Hydrolysis of QM to form BA

The hydrolysis of QM to form BA was studied in unbuffered solutions with pH_{app} 7.11–7.35 and at QM concentrations of (1.75 –4.71) × 10^{-5} M. The addition of water followed simple first-order kinetics with an average value of $k_s = 5.33$ (±0.28) × 10^{-2} h^{-1}. Small changes in the initial concentration of QM and pH had no discernable effect on the kinetics.

The conversion of QM to BA was catalyzed by both the addition of acid and base. The observed first-order rate constants appear to be described by 3, where k_s is the rate constant for the pH-independent addition of water, $k_H[H^+]$ is the contribution of a acid-catalyzed reaction, and $k_{OH}[OH^-]$ is the contribution of a base-catalyzed reaction; k_H and k_{OH} are the corresponding observed second-order rate constants for the acid- and base-catalyzed reactions, respectively.

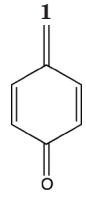
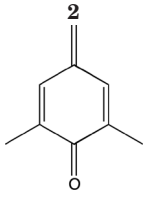
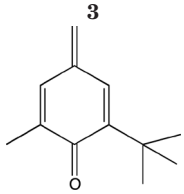
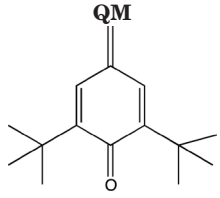
$$k_{obs} = k_s + k_H[H^+] + k_{OH}[OH^-] \quad (3)$$

The acid- and base-catalyzed addition of water to the QM to form BA is a very rapid reaction and was followed by UV–Vis spectroscopy. The reaction followed simple first-order kinetics throughout the acid and the hydroxide concentration range examined, regardless of the counter ion. The data gave an observed second-order rate constant, k_H, of 2.88 (±0.06) × 10^3 M^{-1} h^{-1}, and an observed second-order rate constant, k_{OH}, of 6.42 (±0.16) × 10^2 M^{-1} h^{-1}.

Figure 3 shows the pH_{app}-rate profile for the hydrolysis in the pH_{app} range of 0–14, with the solid line corresponding to that found from a nonlinear regression fit assuming hydrogen ion concentration from Eq. 3 to be $10^{-pH_{app}}$ and hydroxide ion concentration to be $10^{-pKw+pH_{app}}$. Values for the second-order rate constants, $k_H = 1.92$ (±0.13) × 10^3 M^{-1} $h^{-}1$, and $k_{OH} = 2.09$ (±0.20) × 10^2 M^{-1} h^{-1}, and the first-order rate constant, $k_s = 5.11$ (±0.29) × 10^{-2} h^{-1}, are generated from the fit. The differences between these values and the values of k_H and k_{OH} constants, calculated from the plots in Figure 2, reflect the disparity between the concentrations of acid and base added to the acetonitrile–water solvent and those estimated from the pH_{app} values.

At neutral pH_{app} (corresponding to approximately the reaction kinetics carried out in unbuffered solutions), the effective contribution of the acid- and base-catalyzed reactions accounts for less than 0.5% of the observed first-order rate constant, k_s. Therefore, the uncatalyzed addition of water does appear to

Table 2. First- and Second-Order Rate Constants for the Reactions of Various QMs (1–3 and the QM of BHT) in Various Aqueous Solvents

	1	**2**	**3**	**QM**
				
k_s (h^{-1})	1.2×10^{4a}	96^b	53^b	0.18^b 0.053^c
k_H (h^{-1} M^{-1})	1.9×10^{8a}			2.87×10^{3c}

[a]Solvent: water, $I = 0.1$ ($NaClO_4$), 25°C, Ref. 17.
[b]Solvent: acetonitrile–water 20/80, pH 7.4 (phosphate), 25°C, Ref. 16.
[c]Solvent: acetonitrile–water 50/50 (v/v), $I = 0.5$ ($NaClO_4$), this study.
Observed first-order constants reported in h^{-1}, and observed second-order rate constants reported as h^{-1} M^{-1}.

be a significant kinetic pathway consistent with the pH_{app}-rate profile with a plateau from pH_{app} 6–9.

Hydrolysis of QM in the Presence of Chloride Ion

Chloride is a common ion found in many pharmaceutical preparations as an API salt counter ion or used to control the ionic strength of the solution or tonicity in the case of injectable and ophthalmic formulations. An increase in the concentration of sodium chloride from 0.05 to 0.1 M leads to only a small, nonsignificant difference in the observed first-order rate constants for the reaction, $k_{obs} = 4.73 - 4.84 \times 10^{-2}$ h^{-1}. BA is the only product observed and approximate mass balance is maintained.

The observed first-order rate constants are also within about 10% of the rate of the addition of water without added chloride, $k_s = 5.33 \times 10^{-2}$ h^{-1}. Any effect might be attributed to experimental error or at most, a nonspecific ion effect.

Hydrolysis of QM in the Presence of Phosphate Buffer

Phosphate buffers are commonly used in pharmaceutical formulations. Followed by HPLC, the hydrolysis of QM followed first-order kinetics throughout the phosphate buffer concentration range examined with a single product, BA, observed. The pH_{app} values of these reaction solutions at different concentrations of phosphate differ by 0.20 pH_{app} units. Thus, the observed rate constants for phosphate are reported as measured at each pH_{app} value.

An increase in the concentration of phosphate buffer from 0.005 to 0.05 M leads to a small apparent decrease in the observed first-order rate constants for the reaction, $k_{obs} = 5.37 \times 10^{-2}$ to 4.83×10^{-2} h^{-1}. The small effect on rate and the shift in equilibrium of phosphate species, from a change in pH_{app} of 0.20, makes it difficult to generalize about the possible effect of phosphate buffer concentration or the relative reactivity of either phosphate species on the rate of the loss of QM without additional experimentation. Unfortunately, further concentrations and higher pH values for phosphate buffer could not be studied because of the low solubility of phosphate species in 50% (v/v) aqueous acetonitrile.

The observed first-order rate constants are also within 10% of the rate of the addition of water without phosphate buffer present, $k_s = 5.33 \times 10^{-2}$ h^{-1}. This difference is slightly outside the expected error for these measurements. A nonspecific ion effect on the rate for addition of water caused by both phosphate species is possible.

Comparison of QM Reactivity to Other Alkylated QMs

There are some data published on the reactivity at 25°C of structurally similar QMs with water and some nucleophiles.[16–19] Some relevant rate constants for these QMs are shown in Table 2.

The simplest *p*-QM, **1**, was generated by flash photolysis[17] and found to be highly reactive with a half-life of 0.2 s in aqueous solvents. Adding substituents at the second and sixth positions greatly enhance the stability of the QM, as can be seen in the significantly reduced rate constant for the addition of water for **1** and **2,** respectively. The dimethyl (**2**), methyl, *t*-butyl (**3**), and di-*t*-butyl (QM) 2, 6-substituted QMs have a trend of decreasing reactivity with increased size of aliphatic substituents. The addition of a single *t*-butyl group almost doubles the half-life from 26 to 48 s; however, replacing the methyl group of **3** with an additional *t*-butyl substituent increases the half-life 65-fold to 3060 s. The greater enhancement in stability of QM versus QMs **2** and **3** has been attributed to steric hindrance.[16] The large *t*-butyl groups at the second and sixth positions effectively block solvent interactions and, therefore, the stabilization of the partially negative charge on the phenoxyl oxygen. This increases the delocalization of the charge into the QM structure leading to greater stability.

The effect of alkyl phosphates and inorganic phosphate on the rate of disappearance of the QM **2** has been reported in the literature.[19] A stable adduct was observed between **2** and diethyl phosphate in the presence of acid, but it was not specified whether an adduct with inorganic phosphate was observed. However, there was a measurable rate increase. At pH_{app} 7, the observed first-order rate constant from the reaction of **2** in the presence of inorganic phosphate increased 50%.

On the basis of the comparison of the reported rate constants for the addition of water and the observed rate constant in the presence of inorganic phosphate at neutral pH values, a phosphate adduct to QM was not formed to any significant extent in the present study.

Reactivity of QM and Possible *In Vivo* Implications

In addition to QM reacting with nucleophilic functional groups in the drug product, it has also been shown to react with nucleophilic groups found in the body.[16,20–22] The ADI for BHT has recently been raised from 0.05 to 0.25 mg/kg per day, but no similar ADI has been established for QM.[10] The introduction of QM into the body as an oxidation product in a drug product

or through the biotransformation of BHT to QM *in vivo* could lead to adduct formation with biological molecules including DNA.[23,24] The consequences of repeated exposure are unknown but, as discussed in the *Introduction*, this may not be too serious an issue.[10]

CONCLUSIONS

Butylated hydroxytoluene is widely used as an antioxidant in many industries and is present at low levels in several marketed pharmaceutical formulations and some foods. Published data show that BHT is oxidized to QM, which can form adducts with DNA *in* vitro. There is no literature on the reactivity of QM with common nucleophiles present in excipients, API molecules, buffers, and biological molecules present at the site of QM formation.

The reactivity of QM was examined with water in the presence of acid, base, chloride, and phosphate. The reaction with water resulted in a single product, BA. BA is formed through the addition of water to the highly electrophilic methylene carbon of QM. This reaction was catalyzed by the presence of both added acid (H^+) and base (OH^-). However, the presence of added chloride and phosphate had little effect on the reaction kinetics and the product observed at the pH values studied.

The reactivity of QM with a variety of additional nucleophiles should be undertaken. Unpublished data from this study showed the formation of an acetate adduct in acidic acetate buffers. Similar reactivity is expected with other carboxylic acids. An acetate adduct with a reactive QM has been reported elsewhere.[25]

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