# Solid-State Nuclear Magnetic Resonance Determination of the Physical Form of BHA on Common Pharmaceutical Excipients

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***Purpose.*** The purpose of this study was to evaluate the physical form of 2-*tert*-butyl-4-methoxy-phenol (BHA) following wet granulation onto common pharmaceutical excipients.

***Methods.*** A $^{13}C$ label was incorporated into the methoxy group of BHA, the major isomer in synthetic butylated hydroxyanisole. Solutions of the labeled BHA were used to load the labeled BHA onto common pharmaceutical excipients. After air drying under ambient conditions, the mixtures were examined by $^{13}C$ MAS and CP/MAS nuclear magnetic resonance (NMR) spectroscopy to evaluate the physical form of the BHA.

***Results.*** The data suggested that BHA could exist as either a crystalline or an amorphous component and that amorphous material was either bound to excipients or relatively mobile during the time of the NMR experiment. At 0.1% loading, BHA appeared to be amorphous and mobile in the freshly prepared blends. At 0.5% loading, BHA was shown to be amorphous on microcrystalline cellulose (MCC) and hydroxypropylmethylcellulose (HPMC) while remaining crystalline on lactose, mannitol, calcium phosphate dihydrate, and croscarmellose sodium.

***Conclusions.*** Solid-state NMR spectroscopy has been used to probe the physical forms of $^{13}C$-labeled BHA granulated onto common pharmaceutical excipients. The techniques described in this paper may be applied to help explain stability changes in formulations containing BHA.

**KEY WORDS:** amorphous; crystalline; mobile; nuclear magnetic resonance (NMR); 2-*tert*-butyl-4-methoxy-phenol (BHA).

## INTRODUCTION

The use of butylated hydroxyl anisole (BHA) as an antioxidant in solid dosage forms is ubiquitous throughout the pharmaceutical and food industry (1,2). BHA is nearly always evaluated as an antioxidant during the formulation of compounds that are known to degrade by an oxidative mechanism. Unfortunately, the behavior of BHA is frustratingly complex; its ability to successfully retard degradation can vary depending on its concentration, the choice of excipients and processing methods, and the storage conditions (3,4). In some cases, BHA appears to cause oxidation of the drug in certain formulations/conditions while protecting it in others, even at the same BHA loading (3). The lack of techniques to understand the seemingly random behavior often leads teams of pharmaceutical scientists down the resource-intensive pathway of "trial and error."

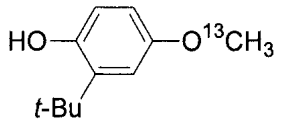
The primary mode of action for BHA is well-known (4,5); it sacrifices itself by donating a hydrogen atom to free radicals, thereby becoming a free radical itself. The BHA radical is stabilized by multiple resonance structures and interferes with the propagation step of the radical reaction, retarding the degradation. The difference between the thermodynamic stability of the BHA and drug-based radicals and the rate of hydrogen atom exchange between them are the major drivers affecting BHA-mediated oxidation rates. Removal of BHA radicals from the system by dimerization or reaction with a reducing agent, such as ascorbic acid, further stabilizes the system.

Despite the significant use of BHA as antioxidant in formulations, its physical state is unclear. Moreover, to the best of our knowledge, no one has previously attempted to correlate the physical state of BHA with the choice of excipients. In solid dosage forms, BHA could be crystalline or amorphous, and the amorphous material could either be "solid-like" or "liquid-like." Because proximity to the drug is an important part of antioxidant activity, it is clear that both the physical form of BHA and its distribution throughout the formulation will be important factors for stabilizing the formulation.

Considering the myriad possible combinations of excipients, processing operations, storage conditions, and BHA loading, a comprehensive study to satisfy the needs of all researchers is beyond the ability of any one study. The primary goal of this paper is to introduce a simple technique for assessing the physical form of the major isomer of BHA in formulations at realistic concentrations. BHA is typically used as a minor component in formulations (0.02–0.5%), which has previously hindered efforts to determine its physical form in such systems. $^{13}C$-labeled BHA can be prepared in a one-step synthesis and $^{13}C$ MAS and CP/MAS nuclear magnetic resonance (NMR) spectroscopy were used to determine the physical form of BHA following wet granulation onto commonly used excipients. The data showed that BHA could exist as a crystalline or an amorphous component and that amorphous material could either be bound to excipients or relatively mobile during the time of the NMR experiment. The chemical shifts of amorphous BHA differ depending on the excipients present, suggesting a large degree of interaction with components of the formulation. Physical form based explanations will be suggested for commonly observed BHA-mediated stability phenomena.

## MATERIALS AND METHODS

Labeled 2-*tert*-butyl-4-methoxy-phenol (3-BHA, Scheme 1) was synthesized by substituting a $^{13}C$-labeled methylating




2-*tert*-butyl-4-($^{13}C$-methoxy)-phenol

**Scheme 1.** Structure of 2-*tert*-butyl-4-($^{13}C$-methoxy)-phenol (3-BHA).

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group into a modified literature procedure for the preparation of 2-BHA (6). $^{13}C_2$-dimethyl sulfate (99% pure, Cambridge Isotope Laboratories, Andover, MA, USA) and *tert*-butylhydroquinone (97% pure, Aldrich, Milwaukee, WI, USA) were combined and dissolved in anhydrous methanol. A solution of NaOH in anhydrous methanol was added dropwise, and the product was isolated and purified using an aqueous work-up followed by flash column chromatography on silica gel using 13% ethylacetate in hexanes as the mobile phase. The pure fractions were crystallized from pentane to yield long white needles that were shown to be 99.5% pure 2-*tert*-butyl-4-($^{13}$C-methoxy)-phenol by high performance liquid chromatography (HPLC) and $^1$H, $^{13}$C NMR as compared to 3-BHA received from Sigma (St. Louis, MO, USA). $^1$H NMR (δ, ppm; $CHCl_3$-d): 1.40 [singlet, C-$(CH_3)_3$], 3.76 (doublet, O-$^{13}CH_3$), 6.59–6.90 (multiplet, CH–CH, C–H). $^{13}$C NMR (δ, ppm; $CHCl_3$-d): 29.1, 34.7, 55.7, 110.5, 114.2, 116.8, 136.7, 148.5, 153.5. M.P. = 61.8°C (DSC).

Excipients used in the study (which are the most commonly used for tablet and capsule formulations of various active pharmaceutical ingredients) included microcrystalline cellulose (MCC) (Avicel PH102, FMC Corporation, Wilmington, DE, USA), mannitol (Pearlitol 200 SD, Roquette America, Keokuk, IA, USA), lactose spray dry NF (Foremost, Rothschild, WI, USA), hydroxypropylmethylcellulose (HPMC 6CPS, Dow, Midland, MI, USA), calcium phosphate dihydrate (Fluka, Fairlawn, NJ, USA), and croscarmellose sodium (FMC Corporation). Mixtures of 3-BHA were prepared by wet-blending solutions of labeled BHA in 9/1 ethanol/water onto the excipients. The mixtures were allowed to air dry at room temperature.

All $^1$H/$^{13}$C CP/MAS and $^{13}$C MAS NMR spectra were obtained on the Bruker (Billerica, MA, USA) DSX-400 NMR spectrometer (9.4-T magnetic field strength) using a Bruker double-resonance CP/MAS probe with standard pulse sequences. The $^{13}$C and $^1$H resonance frequencies are 100.627 MHz and 400.136 MHz, respectively, at this magnetic field strength. $^1$H/$^{13}$C CP/MAS NMR experiments were performed with 2.0 ms contact times, 4K of data points were acquired in 60 ms and then zero-filled to 8K before transformation using 40.0 Hz of line broadening; 1K (K = 1024) scans were acquired. Recycle delays for all NMR experiments were 5.0 s. Rotor frequency was 7.0 kHz. All $^{13}$C spectra were referenced to tetramethylsilane (TMS) using the carbonyl carbon of glycine (176.03 ppm) as a secondary reference.

Differential scanning calorimetry (DSC) analysis was performed using a TA Instruments (New Castle, DE, USA) DSC2920 differential scanning calorimeter. HPLC analysis was performed using a Waters (Milford, MA, USA) Alliance HPLC with a Spherisorb ODS3 column at ambient temperature using a gradient method with 0.1% $H_3PO_4$/acetonitrile and a detection wavelength of 254 nm. The peak retention times were compared to those of a commercial mixture of BHA isomers.

## RESULTS

The $^{13}$C CP/MAS spectra were recorded for all samples containing BHA and for granulated excipients as control studies. With the exception of HPMC, none of the granulated excipients have any peaks or spinning sidebands with chemical shifts between 50 and 60 ppm. The $^{13}$C CP/MAS spectrum of the labeled methoxy group in crystalline BHA is split into three separate peaks with chemical shifts at 55.8, 54.8, and 53.2 as shown Fig. 1A (7). The major forms of BHA as observed by MAS and CP/MAS experiments are summarized in Table I.

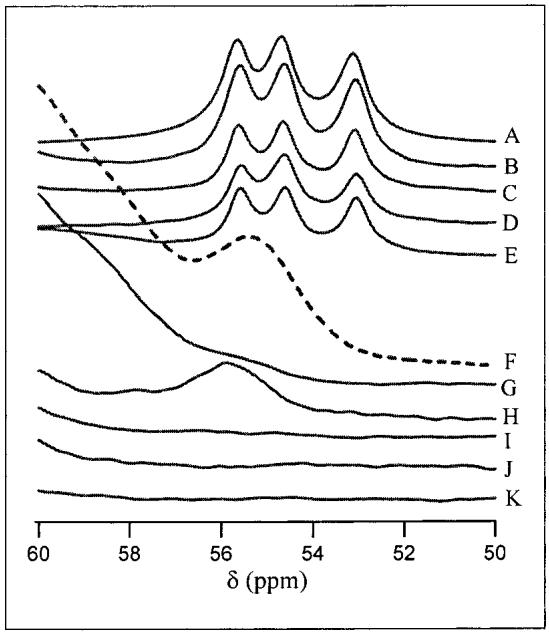


**Fig. 1.** $^{13}$C CP/MAS spectra showing the $^{13}$C-labeled methoxy group of BHA. The samples include (A) bulk BHA, (B) 0.5% BHA on mannitol, (C) 0.5% BHA on lactose, (D) 0.5% BHA on calcium phosphate dihydrate, (E) 0.5% BHA on croscarmellose sodium, (F) 0.5% BHA on HPMC, (G) 0.1% BHA on HPMC, (H) 0.5% BHA on Avicel, (I) 0.1% BHA on Avicel, (J) 0.1% BHA on mannitol, and (K) 0.1% BHA on lactose.

## DISCUSSION

Before we begin our discussion, it is necessary to define the three concepts (crystalline, amorphous, and mobile) that will be used in this paper to define the state of BHA. Because $^{13}$C CP/MAS NMR only detects signal from molecules that possess an unaveraged dipole coupling interaction, only "solid-like" BHA (i.e., either crystalline or amorphous) would yield any signal in this experiment. The loss of signal in the $^1$H/$^{13}$C CP/MAS experiment would suggest that BHA is

**Table I.** Major Form(s) of BHA on Excipients at Various Loading Levels Based on $^{13}$C MAS and CP/MAS

| Excipient | Percentage BHA by weight | Physical form[a] |
|---|---|---|
| Mannitol | 0.5% | Crystalline |
| | 0.1% | Mobile |
| Lactose | 0.5% | Crystalline |
| | 0.1% | Mobile |
| $CaHPO_4$–$2H_2O$ | 0.5% | Crystalline |
| Croscarmellose sodium | 0.5% | Crystalline |
| Avicel | 0.5% | Amorphous |
| | 0.1% | Mobile |
| HPMC | 0.5% | Amorphous |
| | 0.1% | Mobile |

[a] Only the predominant form is depicted. All mixtures showed varying amounts of other forms present. See text for details.

in a mobile state (i.e., "liquid-like"). In order to observe the mobile components, the $^{13}C$ MAS spectra must be acquired as well (8,9).

The $^{13}C$ CP/MAS NMR spectra showed that the three peaks corresponding to crystalline 3-BHA are preserved at 0.5% loading on mannitol, lactose, croscarmellose sodium, and calcium phosphate dihydrate (Fig. 1 B to E), suggesting that BHA crystallizes on these excipients. The three peaks are replaced by a single broad peak at 0.5% loading onto either MCC or HPMC (Fig. 1 F to H), indicating that the BHA remains amorphous (but "solid-like") when deposited onto these excipients. The NMR signal vanishes when the loading of BHA on Avicel, mannitol, or lactose is lowered from 0.5 to 0.1% (Fig. 1 I to K**).**

Comparison of $^{1}H/^{13}C$ CP/MAS and $^{13}C$ MAS experiments may provide information on the presence of mobile (or "liquid-like") BHA. The two spectra were compared for each mixture in order to determine whether or not there was a significant mobile BHA component in the mixture. Figure 2 shows overlays of the spectra taken for bulk 3-BHA and the mixtures containing 0.5% BHA on lactose, calcium phosphate, and croscarmellose sodium. The $^{13}C$ MAS NMR spectrum of bulk crystalline BHA contains the same three peaks in roughly the same ratio observed by the CP/MAS technique. CP/MAS spectra of crystalline BHA in the mixtures appear similar to the spectrum of bulk BHA, but distinct changes are visible in their $^{13}C$ MAS spectra. The crystalline peak at δ53.2 ppm decreases in intensity relative to the peaks at δ55.8 and 54.8 ppm, and the resolution of the downfield peaks decreases as if a broad peak is increasing in intensity beneath them. The effect is observed in all of the mixtures to differing degrees suggesting that the mixtures contain both crystalline and mobile BHA. No attempts have been made to quantitate the fractions of mobile and crystalline BHA at this time.

$^{13}C$ MAS spectra of samples containing 0.1% BHA on MCC, HPMC, mannitol, and lactose which appeared amorphous or gave no observable signal by $^{13}C$ CP/MAS spectroscopy are shown in Fig. 3. The presence of a signal in $^{13}C$ MAS and the absence of signals in $^{13}C$ CP/MAS suggests that BHA is in a mobile form. The observed mobility could result from either a low affinity of the crystalline surfaces for BHA or from insufficient excipient surface area to bind a spectroscopically observable quantity of substrate.

Amorphous BHA is observed at different chemical shifts on each excipient, whether it is mobile or not. The most likely cause for the chemical shift changes is interaction with the excipients, analogous to solvent-dependent chemical shifts common in solution NMR. The $^{13}C$ MAS spectra of samples containing 0.1% BHA on lactose and mannitol show at least two broad overlapping peaks demonstrating that BHA experiences multiple chemical environments. Possible environments include those in which BHA interacts with excipients and those where it does not. Indeed, the related 3,5-di-*t*-butyl-4-hydroxyanisole is known to crystallize through intermolecular hydrogen-bonds using the phenol as an H-bond donor and the methoxy as an H-bond acceptor (10). Lactose and mannitol are both composed of primary and secondary alcohols, and they are both known to have amorphous components (11). Considering the myriad hydrogen-bond donors and acceptors present and the possible contribution of "free" BHA, it is quite reasonable that multiple broad peaks were observed (12). However, the fact that the signals are only observed by $^{13}C$ MAS spectroscopy suggests that the interactions are facile.

The beneficial effects of BHA typically plateau or even decrease at a loading that is between 0.1% and 0.5% BHA depending on the drug, the formulation, and the storage conditions. It is not unusual for formulations containing excess BHA to degrade much faster than those with none at all (3).

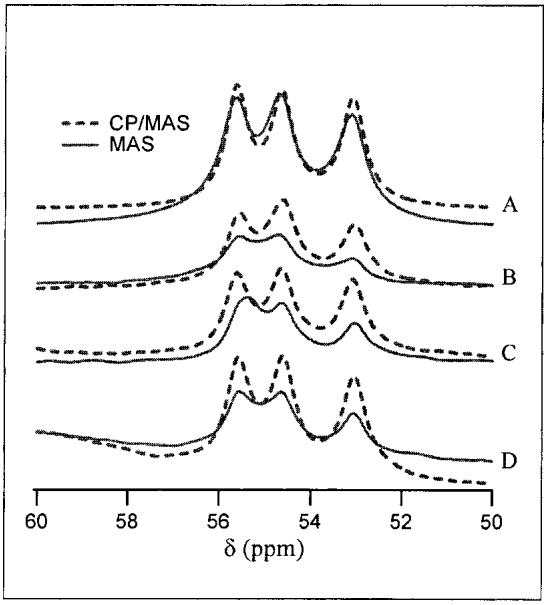


**Fig. 2.** Comparison of $^{13}C$ MAS and $^{1}H/^{13}C$ CP/MAS spectra of (A) bulk 3-BHA, (B) 0.5% BHA on calcium phosphate dihydrate, (C) 0.5% BHA on lactose, and (D) 0.5% BHA on croscarmellose sodium.

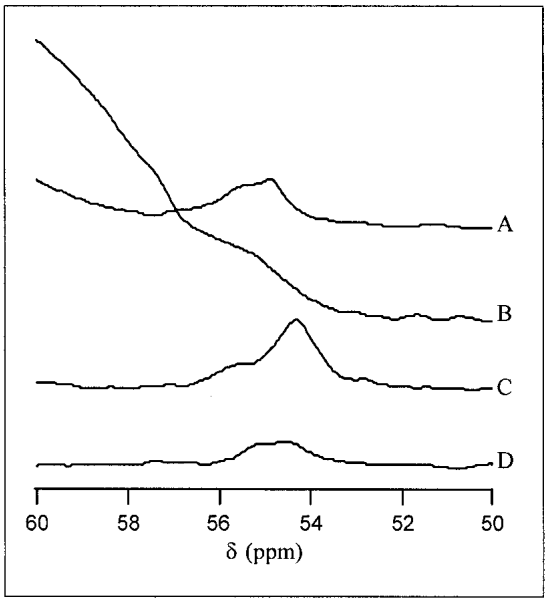


**Fig. 3.** $^{13}C$ MAS spectra of samples that appeared amorphous or gave no observable signal when measured by $^{13}C$ CP/MAS spectroscopy. The samples contain (A) 0.1% BHA on Avicel, (B) 0.1% BHA on HPMC, (C) 0.1% BHA on mannitol, and (D) 0.1% BHA on lactose.

In many formulations, BHA extends the initiation period, staving off oxidation for a period of time that is difficult to predict. Traditional explanations have included the slow loss of BHA by sublimation and reaction with radicals and by the slow accumulation of radicals that eventually overwhelm the antioxidant. These reasons are important and must be considered carefully when working to stabilize a formulated compound. However, the physical state of the BHA should also be considered when erratic behavior is encountered. The fact that BHA crystallizes on numerous excipients at 0.5% loading within 1 day of drying suggests a reason for plateauing or decreasing antioxidant activity: crystalline BHA is thermodynamically more stable and essentially immobile. Moreover, because the radical form is less likely to be included as BHA crystallizes, the proportion of mobile radical/nonradical could increase sufficiently to favor initiation.

The issue of the induction period may also have a physical form component in some cases. Once BHA crystals nucleate and begin to grow, they could become a thermodynamic sink to which mobile BHA will continue to migrate until an equilibrium is reached. The rate of nucleation could drop off rapidly as BHA concentration is reduced. Only long-term detailed investigations into many systems will be able to determine the impact of BHA physical form issues in formulation stability. While many questions remain unanswered, it is our hope that we and others will be able to apply the techniques, results, and hypotheses in this paper to the more rapid development of formulations that are stable and well understood.

## REFERENCES

1. H. Verhagen, P. L. Schilderman, and J. C. S. Kleinjans. Butylated hydroxyanisole in perspective. *Chem. Biol. Interact.* **80**:109–134 (1991) and references therein.
2. J. W. Finley and J. R. Given. Technological necessity of antioxidants in the food industry. *Food Chem. Toxicol.* **24**:999–1006 (1986).
3. T. R. Kommuru, M. Ashraf, M. A. Khan, and I. K. Reddy. Stability and bioequivalence studies of two marketed formulations of coenzyme Q10 in beagle dogs. *Chem. Pharm. Bull.* **47**:1024–1028 (1999).
4. L. Chalmers. Antioxidants: research and development report. *Soap Perfum. Cosmet.* **44**:29–38 (1971).
5. L. R. Mahoney. Antioxidants. *Angew Chem. Int. Ed. Engl.* **8**:547–555 (1969).
6. L. K. Lam and K. Farhat. The use of pivaloyl chloride in the selective synthesis of 3-tert-butyl-4-methoxyphenol—a BHA isomer. *Org. Prep. Proceed. Int.* **10**:79–82 (1978).
7. Since there is only one labeled carbon in the BHA and one isomer of BHA present, the three peaks were attributed to the existence of multiple molecules per unit cell. Differential scanning calorimetry, DSC, shows only one transition, an endotherm at 61.8 °C with $\Delta H$ of 120.6 J/g.
8. C. A. Fyfe. *Solid State NMR for Chemists*. CFC Press, Guelph, Ontario, Canada, 1984.
9. R. Voelkel. High-resolution solid-state 13C NMR spectroscopy of polymers. *Angew. Chem. Int. Ed. Engl.* **27**:1468–1483 (1988).
10. Structures of phenols were found in the Cambridge Structural Database using ConQuest v. 1.5 and visualized using Mercury v. 1.1.1.
11. Handbook of Pharm. Excipients 2000, In A. H. Kibbe (ed). 3rd ed., American Pharmaceutical Association, 2000, pp. 276–285, 324–327.
12. Lactose is known to consist of varying amounts of an amorphous component depending on processing and storage conditions, and the two peaks could also result from a facile interaction with the amorphous and crystalline phases, or even from BHA that is "dissolved" in amorphous lactose vs. BHA that is not. Excipient-grade mannitol typically contains small amounts of sorbitol that are believed to reside as an amorphous coating on the crystals, thereby providing beneficial processing characteristics. It is possible that one of the BHA peaks on mannitol results from interaction with this layer.