



Confounding contaminants in mass spectrometric reaction monitoring

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ABSTRACT

Reactions carried out in the presence of rubber septa run the risk of additives being leached out by the solvent. Normally, such species are present at low enough levels that they do not interfere with the reaction significantly. However, when studying reactions using sensitive methods such as mass spectrometry, the appearance of even trace amounts of material can confuse dynamic analyses of reactions. A wide variety of additives are present in rubber along with the polymer: antioxidants, dyes, detergent, and vulcanization agents, and these are all especially problematic in negative ion mode. A redesigned Schlenk flask for pressurized sample infusion (PSI) is presented as a means of practically eliminating the presence of contaminants during reaction analyses.

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1. Introduction

Pressurized sample infusion (PSI) [1,2] is an effective way to analyze chemical reactions in real time using ESI-MS as it allows for monitoring the behaviour of low-intensity catalytic species as they are produced. We (and others) have used PSI to study a wide range of catalytic reactions in both positive [3–15] and negative ion mode [16–18]. In these experiments, impurities are not uncommon, but are generally present either from the start of the reaction or appear upon addition of a particular reaction component, and do not change significantly in intensity over the course of a reaction [19]. However, we have also observed a number of unexpected/unidentified ions have been observed growing more abundant over the length of an experiment in negative ion mode. The m/z values of these unexpected ions tend to be found in the lower mass region of the spectrum, and can overlap and/or obscure the low-intensity species of interest. Even at low levels, such contaminants can complicate mechanistic analyses because they do not align with the predicted species based on the chemicals present, and because their dynamics can match those expected of decomposition products. Such decomposition products are of interest because they can often suggest ways in which the catalyst is dying,

and therefore knowing the source of a particular signal is important. Both low resolution [20] and high resolution [21] mass spectrometry can provide structural information needed to identify unknown species, but avoiding contaminants in the first place is always the best practice

Previous mass spectrometric research has reported vulcanizing agents and antioxidants leaching into reaction mixtures [22–25]. The incidental contact of solvent to rubber septa results in the leaching of interfering ions into the reaction mixture, and this process is especially efficient when the reaction solvent is heated. Primary and secondary antioxidants are often used to prolong or inhibit the free radical chain mechanism of oxidative decomposition in rubber, plastics, and foods. Primary antioxidants can disrupt autoxidation by neutralizing free radicals active in the propagation step via donation of hydrogen atoms to these free radicals [26]. In phenolic antioxidants this occurs via hydrogen-atom transfer (HAT) when the bond dissociation enthalpy of the O–H bond is low [27,28]. Alternatively, these antioxidants can perform a single electron transfer (SET) to a free radical, and it is likely that both HAT and SET occur in parallel [27,28]. In comparison, secondary antioxidants react directly with the oxidizing agents and are regularly utilized in tandem with primary antioxidants [29,30]. Alkylated phenols have long been known to stabilize polymers and to prevent oxidation of natural rubber after vulcanization [31–34]. In order for a given phenol to act as an effective primary antioxidant, the alcohol group must be in a chemical environment in which the acidic hydrogen

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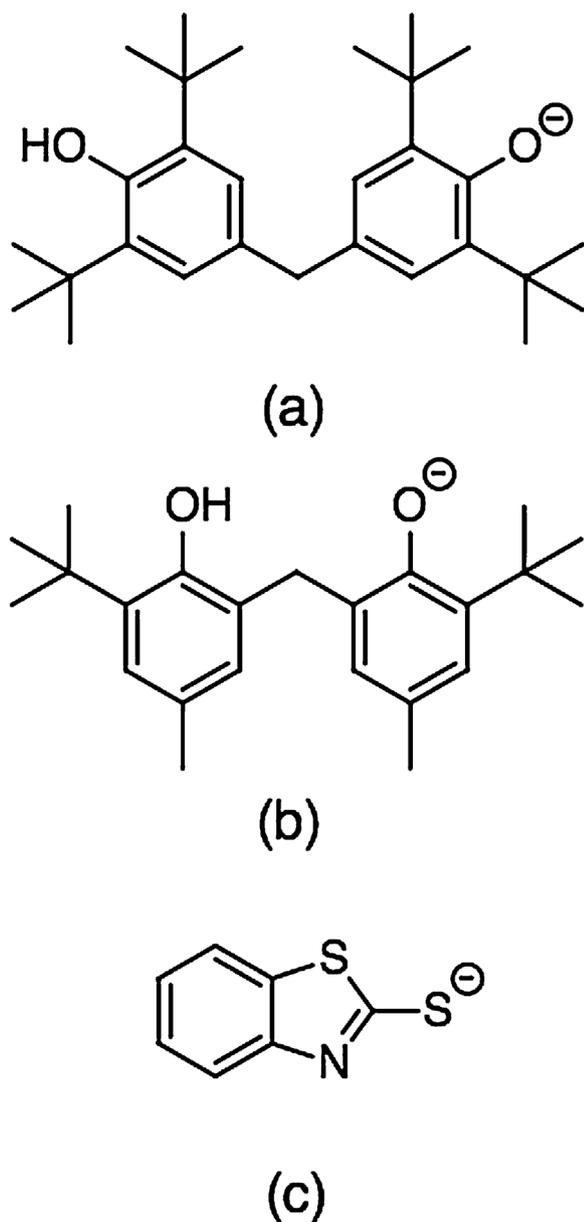


Fig. 1. (a) 4,4'-methylenebis(2,6-di-tert-butylphenol) ($[M-H]^-$ at m/z 423, **1**), (b) 2,2'-methylenebis(4-methyl-6-tert-butylphenol) ($[M-H]^-$ at m/z 339, **2**), and (c) 2-mercaptobenzothiazole ($[M-H]^-$ at m/z 165, **3**).

of the phenol is readily available for donation, allowing for free radical neutralization. Additionally, the reactivity of the phenoxy radical intermediate must be minimized to prevent decomposition of the antioxidant [30]. In most phenolic antioxidants, these requirements have been satisfied by the introduction of steric bulk in the ortho position relative to the alcohol group, which helps to stabilize the phenoxy radical intermediates and increases the antioxidation potency of the phenols.

Mercaptobenzothiazoles are used to accelerate the vulcanization of rubber [35], inhibit corrosion [36], as herbicides, as anti-tumor agents [37], and are present in food storage containers with septa [38,39]. The sulfur component is able to facilitate cross-linking via various mechanisms [40], which ultimately hardens the rubber. Rubber septa are widely known to be vulcanized, and as such vulcanizing agents may be observed in reactions where contact has occurred between the solvent and septum.

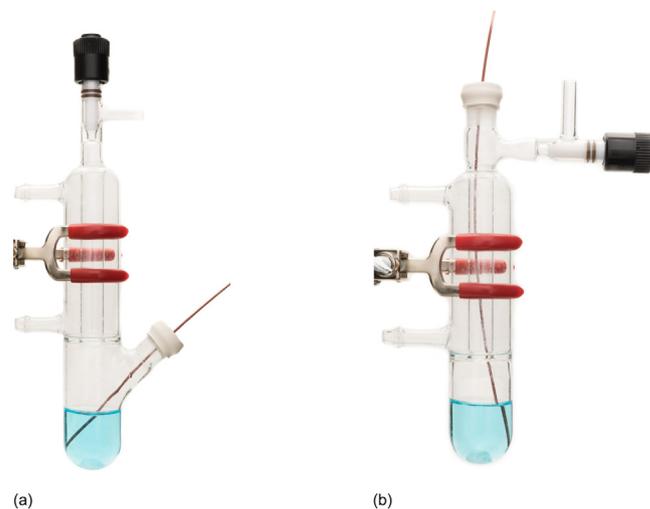


Fig. 2. (a) First generation PSI flask; (b) Re-designed second generation PSI flask with ground glass joint above the condenser, positioned adjacent to the gas inlet tap.

Herein we identify contaminants originating from vulcanization and antioxidant additives in rubber septa, and we present a simple redesign of the glassware used to monitor reactions at elevated temperature, which prevents these contaminants from entering a reaction.

2. Experimental

Reagent grade acetonitrile (Caledon Laboratories, Georgetown, ON, Canada) and methylene chloride (Fisher Chemical, Hampton, NH, USA), and HPLC-grade methanol (Fisher Chemical) solvents were purchased, and used without further purification. Natural rubber septa were purchased from VWR (Radner, PA, USA) (Cat No. 89097-554). Benzoic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in the solvent being tested (i.e. acetonitrile, methanol, or dichloromethane). All mass spectra were collected on a Waters Acquity Triple Quadrupole Detector (Waters Corporation, Milford, MA, USA) in negative ion mode, with the following electrospray ionization parameters: capillary voltage, 3000 V; cone voltage, 10 V; extraction voltage, 3 V; source temperature, 80 °C; desolvation temperature, 210 °C; cone gas flow, 100 L/hr; desolvation gas flow, 500 L/hr; collision voltage, 2 V.

Benzoic acid stock solutions (100 μ M) were made in each of the three solvents. In each experiment 0.5 mL was injected into 10 mL of solvent, as an internal standard. A PSI flask (Schlenk flask equipped with a built-in condenser) was equipped with a stir bar and a septum. PEEK tubing was inserted through the septum into the solution on one end with the other end attached to the ESI source of the mass spectrometer. Argon gas was introduced to the PSI flask at 3 psi whereby the positive pressure forced the solution out of the flask through the tubing to the mass spectrometer. The solution was gradually heated in an oil bath for up to 45 min, SRM spectra of each m/z value were recorded once per second.

High-resolution accurate mass analyses were then obtained on an LTQ Orbitrap VelosTM instrument (Thermo Scientific, Waltham, MA, USA) using a full scan in negative ion mode (m/z 100–1000). ESI-MS was carried out via direct infusion of 500 μ L of the sample was facilitated at 30 μ L/min in methanol using a syringe pump. The spray voltage was set to 2500 V, the capillary temperature was maintained at 250 °C, the sheath gas flow rate was 15, the aux gas was set to 5, and the sweep gas was at 0.

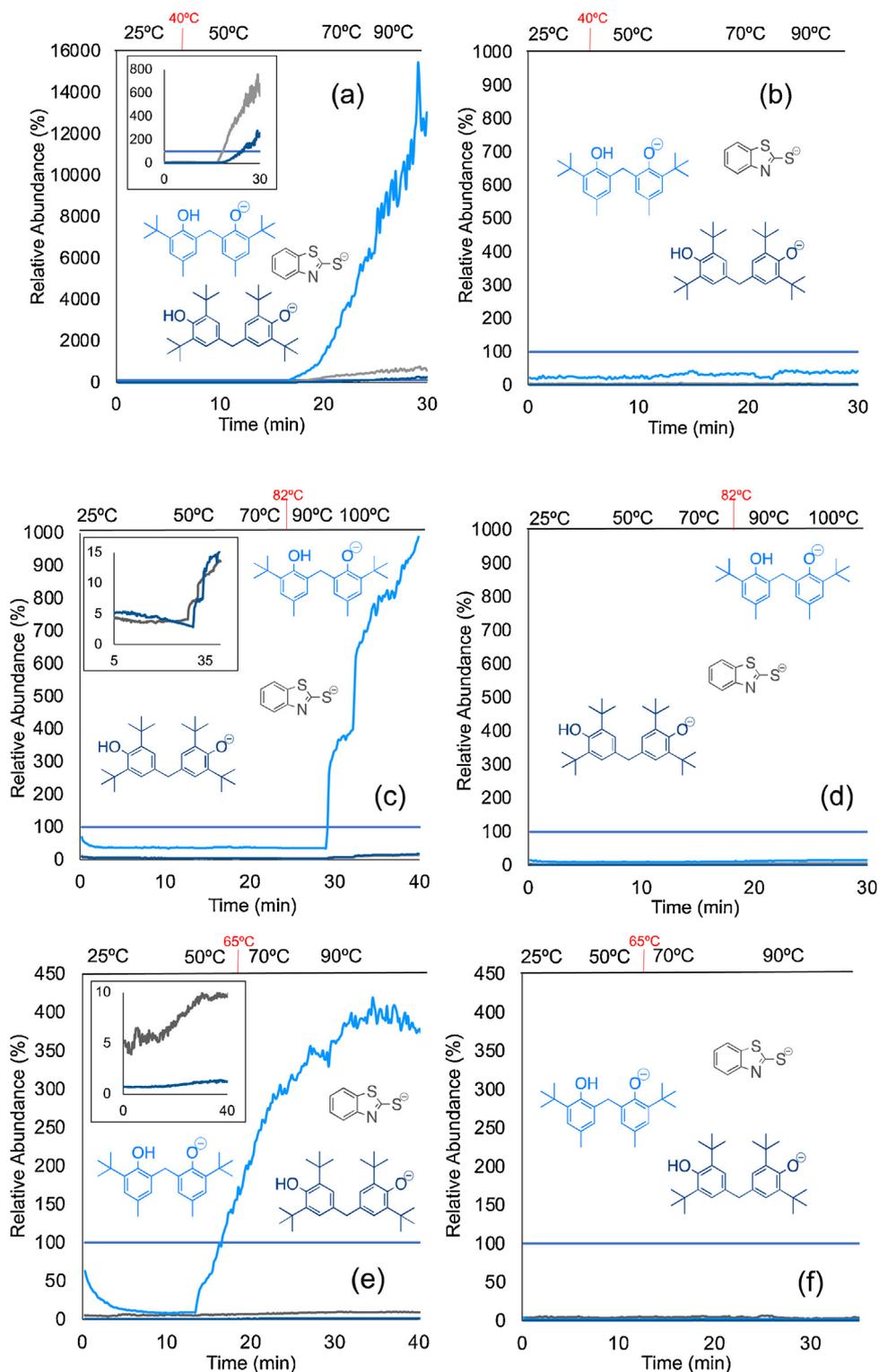


Fig. 3. Monitoring the relative abundance of **1** (m/z 423), **2** (m/z 339), and **3** (m/z 165) at various temperatures (T reported is bath temperature, so values over the b.p. indicate more vigorous refluxing) over time. Benzoic acid was used as an internal standard and as such is observed at 100% relative abundance. (a) Dichloromethane solvent, septum placement below the condenser; (b) dichloromethane solvent, septum placement above the condenser; (c) acetonitrile solvent, septum placement below the condenser; (d) acetonitrile solvent, septum placement above the condenser; (e) methanol solvent, septum placement below the condenser; (f) methanol solvent, septum placement above the condenser.

3. Results and discussion

Natural rubber septa are present in most lab environments, and are often marketed for facile chemical addition to an air-free environment. In this study, VWR natural rubber sleeve stoppers were

used to demonstrate the increasing concentration of vulcanizing agents and antioxidants extracted from a rubber septum by hot solvent. As mass spectrometry is an extremely sensitive analytical technique, even low concentrations of these compounds can interfere with analysis.

We identified three unknown species observed in our mass spectra: 4,4'-methylenebis(2,6-di-tert-butylphenol) ($[M-H]^-$ at m/z 423, **1**) and 2,2'-methylenebis(4-methyl-6-tert-butylphenol) ($[M-H]^-$ at m/z 339, **2**) as antioxidants, and 2-mercaptobenzothiazole ($[M-H]^-$ at m/z 165, **3**) as a vulcanizing agent (Fig. 1), all extracted from natural rubber septa. The growing presence of these compounds was observed using low-resolution mass spectrometry, and their identities were then confirmed with high-resolution accurate mass data (Table S1) [21].

The exact mass of m/z 339.23245 provided by the orbitrap mass spectrometer corresponded to only one CHNO ion to within 1 ppm: $C_{23}H_{31}O_2$ [41]. As operation was in negative ion mode this species is assumed to be deprotonated, giving the formula $C_{23}H_{32}O_2$. This formulation includes an acidic proton as well as unsaturation to the tune of eight double bond equivalents (DBE), thus pointed to an aromatic compound (two benzene rings is eight DBE). A phenol derivative was strongly indicated as it would account for high ionization efficiency in negative ion mode. A search revealed **2** (also known as antioxidant 2246) as the most likely candidate (Fig. 1b). This result guided our assignment of m/z 423.32631 as **1**, another structurally related antioxidant (Fig. 1a) [34,41].

A single result carrying the same isotopic pattern observed in the spectrum was obtained within 1 ppm for the observed m/z 165.97802: $C_7H_4NS_2$. As deprotonation was assumed in negative ion mode analysis, the molecular formula was determined to be $C_7H_5NS_2$. This formula also includes an acidic proton, and six degrees of unsaturation. **3** was found to meet these requirements, and is a known vulcanizing agent (Fig. 1c) [36].

The additive compounds we identified were observed throughout various PSI experiments using a first generation PSI flask. This flask was designed with a tap above the condenser to control the inlet of inert gas, and a ground glass joint covered by a septum below the condenser (Fig. 2a). As the joint was below the condenser, the septum was in close proximity to the reaction mixture and within reach of volatile solvent. The concentration of contaminants is proportional to the temperature of the reaction, however the quantity of each is dependent on the solvent. Dichloromethane was found to extract the highest concentration of **1**, **2** and **3** (Fig. 3a). In comparison, the concentration of these additive was significantly lower in acetonitrile (Fig. 3c), and methanol was found to extract the lowest quantity (Fig. 3e). Signal intensity for the observed contaminants would likely increase in the presence of a deprotonating agent as ionization would be improved [42].

In order to prevent **1**, **2** and **3** from entering the reaction vessel, a second generation PSI flask was created. This model is equipped with a rubber septum above the condenser, adjacent to the gas inlet tap, to ensure that volatile solvent cannot make contact with the rubber. The only consequence of this design is the increased length of capillary tubing leading to the mass spectrometer (Fig. 2b). However, the second generation flask design has been shown to successfully eliminate contamination from rubber septa in all three solvents (Fig. 3cdf).

4. Conclusion

Overall, three frequently observed unknown peaks were identified as 4,4'-methylenebis(2,6-di-tert-butylphenol), 2,2'-methylenebis(4-methyl-6-tert-butylphenol), and 2-mercaptobenzothiazole. As all have a known presence in rubber septa, the PSI flask used to study reactions in real-time was redesigned to prevent these contaminants from entering the reaction. By positioning the septum above the condenser and away from the main reaction vessel, hot solvent no longer has the ability to extract additives from the rubber. This design has proven to

eliminate contamination, and results in spectra free of extraneous contributions from species unrelated to the reaction under study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijms.2019.04.001>.

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