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Understanding and Control of Dimethyl Sulfate in a Manufacturing Process: Kinetic Modeling of a Fischer Esterification Catalyzed by H_2SO_4

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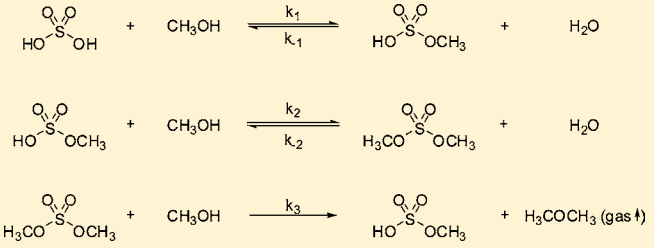
Supporting Information

ABSTRACT: The formation and fate of monomethyl sulfate (MMS) and dimethyl sulfate (DMS) were studied by proton NMR for a sulfuric acid catalyzed esterification reaction in methanol. The kinetic rate constants for DMS and MMS were determined at 65 °C by fitting time-dependent experimental data to a model using *DynoChem*. In refluxing methanol, sulfuric acid was converted to monomethyl sulfate (MMS) in nearly quantitative yield within 45 min. Once formed, the MMS underwent a reversible esterification reaction to form DMS. Dimethylsulfate reacted with methanol to regenerate MMS and form dimethyl ether. A byproduct of the esterification reaction was water, which further consumed DMS through hydrolysis. On the basis of derived rate constants, in refluxing methanol, DMS would not be expected to exceed 4 ppm in the reaction mixture at equilibrium. In the presence of the carboxylic acid substrate, DMS was not detected in the reaction mixture. The reaction pathways of this system have been systematically investigated, and the results of this study will be presented.



INTRODUCTION

There is significant interest in the pharmaceutical manufacturing and regulatory communities regarding the generation and analysis of genotoxic impurities.[1] In 2010, 26 articles were published that highlighted the phrase "genotoxic impurities" as a keyword.[2] We received the following inquiry during regulatory review of a commercial process to manufacture a drug candidate: "Based on the synthetic pathway, including purification steps, of *the drug substance,* please address the potential formation of methyl hydrogen sulfate and dimethyl sulfate impurities with a validated analytical method, which is sensitive enough to detect and quantitate the potential genotoxic impurities. The maximum daily intake of the potential genotoxic impurities should not exceed 1.5 μg per person per day by taking the highest dose of *your clinical candidate.*"

The process in question consisted of the sulfuric acid-catalyzed esterification of a dicarboxylic acid in methanol. A large body of literature describes dimethyl sulfate (DMS) as a known genotoxin; furthermore, its reactivity as an electrophilic methylating agent in S_N2 alkylations is greater than that of methyl iodide.[3] In contrast, monomethyl sulfate (MMS) is a poor alkylating agent and is not genotoxic.[3] The potential formation of DMS as an impurity in the reaction matrix thus became the primary focus of our investigation.

Commercial DMS manufacture is typically performed with SO_3 and anhydrous methanol catalyzed by Pd or other transition metals.[3] These forcing conditions are quite different than the gentle reflux of H_2SO_4 in methanol encountered during the esterification process used to make the active pharmaceutical ingredient (API). The question posed by the regulator was easily addressed with a direct analysis method using gas chromatography (GC) to measure dimethyl sulfate in the isolated drug substance. Multiple batches produced at commercial scale were tested with a validated GC method (limit of detection, LOD = 0.1 ppm), and DMS was not observed in the API.

From a quality-by-design (QbD) perspective, the more relevant technical question for the chemistry team was "could DMS be generated in the reaction mixture prior to API isolation?"

The GC method used to analyze isolated drug substance could not be applied to in-process solutions that contained sulfuric acid, carboxylic acid ester, and methanol. Detailed method development efforts revealed that DMS could actually be formed in the heated injection port when samples of H_2SO_4 in methanol were subjected to GC analysis. It was not possible at that time to successfully mitigate artifacts arising from the sample matrix, and HPLC methods lacked the necessary sensitivity and selectivity.

An orthogonal analytical method for DMS was also investigated using a well-known derivatizing agent, triethylamine (TEA), followed by HPLC–MS analysis.[4] Unfortunately, it was

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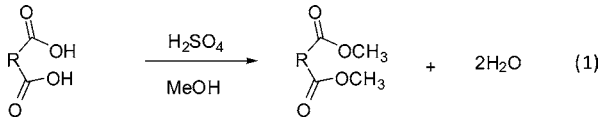
discovered that the diester drug substance also reacted with triethylamine, producing a false positive result for DMS.

Around this time, an investigation was published that described the use of GC–MS to measure reaction kinetics for the formation of methyl methanesulfonate.[5] This compound is also a genotoxic impurity and of regulatory interest because of its potential to form under conditions used to manufacture mesylate salts of an API.

Herein, we discuss the application of ^{1}H NMR techniques and kinetic modeling to the complex multistep equilibria of dimethyl sulfate and monomethyl sulfate under reaction conditions used to manufacture the drug substance.

RESULTS AND DISCUSSION

This investigation was undertaken to better understand the potential to form sulfuric acid-derived impurities in a commercial diester synthesis. The reaction employs methanol and catalytic amounts of sulfuric acid to effect esterification of the dicarboxylic acid starting material (eq 1). Sulfuric acid-derived reaction byproduct could potentially include monomethyl sulfate and dimethyl sulfate.



(1)

The esterification reaction kinetics were first examined to establish the baseline conditions needed to further study formation of the two sulfate ester impurities (MMS, DMS). The reaction mixture consisted of 1 equiv dicarboxylic acid, 16.5 equiv methanol (5.7 volumes), and 0.25 equiv concentrated H_2SO_4 (0.12 volumes). The solution was heated to a gentle reflux at 65 °C, and time-course samples were analyzed by ^{1}H NMR. The reaction was complete within 3 h, and integral peak data were used to produce the reaction kinetic profile presented in Figure 1 (millimoles vs time).

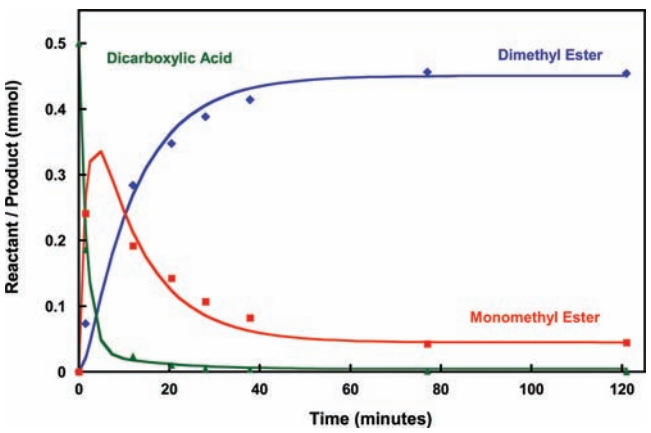
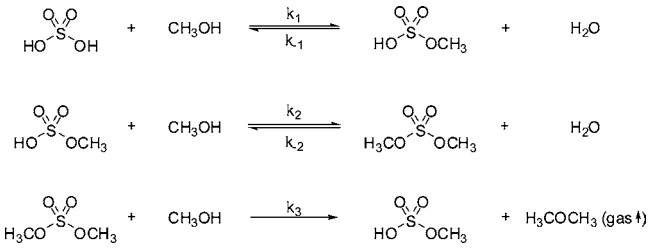


Figure 1. Reaction profile for the esterification of a dicarboxylic acid by methanol and sulfuric acid as measured by NMR. Experimental data represented by individual points on the graph.

The NMR-derived data in Figure 1 were consistent with lab- and production-scale sampling studies where the esterification reaction was >95% complete within 1 h at 65 °C. Subsequent kinetic studies focused on generation and consumption of the two sulfate esters and completed within 60 min.

A multistep reaction mechanism based on published work[6] is proposed in Scheme 1 for the generation and consumption of MMS and DMS.

Scheme 1. Proposed pathways for the formation and degradation of DMS and MMS

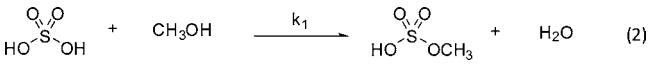


In contrast to previously studied alkylsulfonic acids (such as methanesulfonic acid and ethanesulfonic acid), sulfuric acid can undergo sequential reactions with methanol to generate two different sulfate esters. Monomethyl sulfate is a relatively benign, nongenotoxic impurity that can be controlled like other process-related contaminants.[3] Conversely, DMS is a known genotoxic species and must be controlled to very low levels.[7] It was important to examine the formation and fate of these two sulfate esters under actual process conditions to fully understand the potential process risk presented by DMS.

In order to make the study of this complex multistep mechanism manageable, the pathway was broken into four discrete steps, and the results of each were used to develop a comprehensive kinetic model. Where possible, a rate constant was independently evaluated and determined. The effect of water concentration on each corresponding rate constant was separately evaluated and incorporated into the final model. Results from each step allowed an overall mechanistic model to be constructed. Early kinetic studies were conducted without the dicarboxylic acid substrate, but it was included in subsequent investigations.

Monomethyl Sulfate: Rate of Formation and Degradation. The initial sets of experiments were designed to investigate the formation (k_1) and degradation (k_{-1}) of monomethyl sulfate, the key intermediate in the pathway to the formation of dimethyl sulfate.

1. Formation of Monomethylsulfate (k_1). The formation of MMS is depicted in eq 2:



(2)

Dry methanol (<0.01% water) was mixed with concentrated, dry sulfuric acid and heated to 65 °C. After waiting one minute to reach temperature, seven discrete samples were collected within 12 min and ^{1}H NMR data (16 transients) obtained for these solutions. Equilibrium was reached within 1 h, resulting in an essentially quantitative conversion of H_2SO_4 into monomethyl sulfate. The heated sample remained unchanged after several days' storage in a sealed tube. Identity of the MMS resonance in the NMR spectrum was confirmed by spiking an authentic sample of monomethyl sulfate into the reaction mixture. The MMS peak integral ($C\underline{H}_3$, 3.45 ppm) was normalized to the methyl peak resonance of the methanol solvent ($C\underline{H}_3$, 3.18 ppm) and *DynoChem*[8] used to calculate the forward rate constant (k_1) from the peak-integral data. The study was

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conducted twice with good agreement between the two derived rate constants. The experimental (diamonds) and fitted (line) data for the formation of monomethyl sulfate are presented in panel a of Figure 2 (early time points in reaction) and panel b

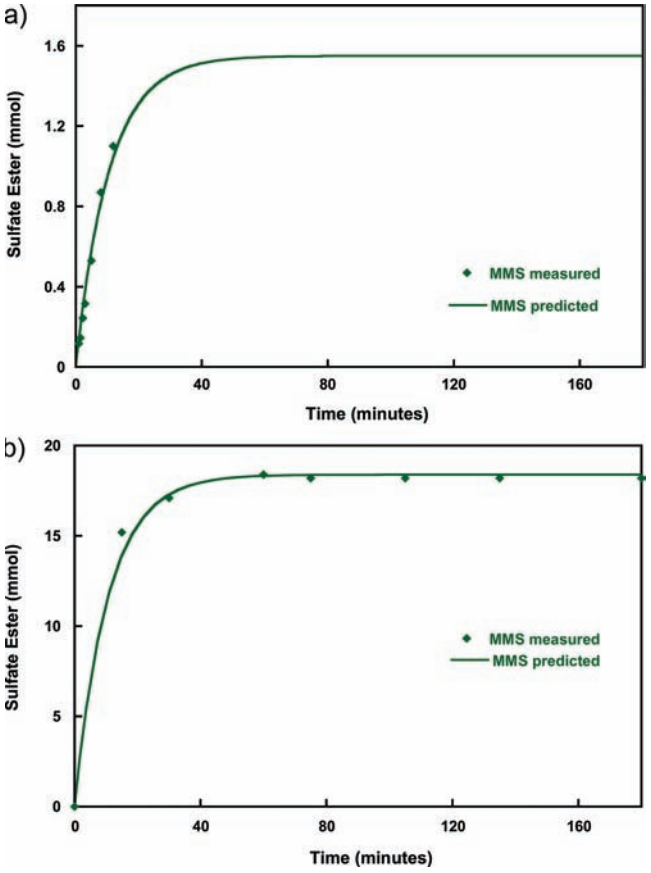
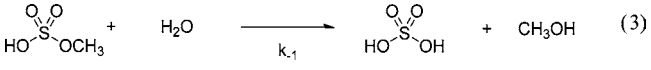


Figure 2. Experimental and predicted fit of data for the formation of monomethyl sulfate at 65 °C. (a) Data points collected in the first 12 min, 0.379 M H_2SO_4. (b) Equilibrium data points obtained from 15 to 180 min, 0.365 M H_2SO_4.

of Figure 2 (later reaction time points). The H_2SO_4 concentrations were similar for both studies but they were run at different scales (5 mL vs 50 mL). The second-order forward rate constant (k_1) for the formation of MMS at 65 °C was experimentally determined to be 6.5×10^{-5} L/mol s with a confidence interval of ±7% RSD.

2. Hydrolysis of Monomethylsulfate (k_{-1}). As illustrated in Scheme 1, monomethyl sulfate can either hydrolyze back to sulfuric acid (k_{-1}) or further react with methanol to form DMS (k_2). The hydrolysis of MMS is presented in eq 3



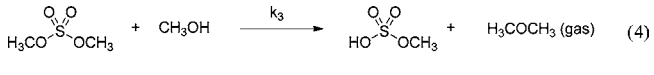
(3)

The rate constant of this reaction was measured by spiking water into solutions containing MMS (1.5 mol %) and monitoring the heated, sealed vessel by ^{1}H NMR for 45 h. Water was spiked into the matrix at two different levels, −6 and 12 mol %. In both cases, the MMS level remained virtually unchanged, confirming that the equilibrium for this reaction lies far to the right (eq 3). In order to develop the larger model, the MMS equilibrium constant $K = (k_1/k_{-1})$ was assigned a value of 999:1 in favor of the forward reaction, thus conservatively defining k_{-1} as 6.5×10^{-8} L/mol s.

In related work, Wolfenden and Yuan[9] measured the rate constants for the hydrolysis of MMS in water over a range of temperatures and pH and found the extrapolated value (at 25 °C) to be 1.7×10^{-8} L/mol s (1 M HCl, T = 40−100 °C).

Results from both laboratories agree that MMS is rapidly formed and stable over a wide range of temperatures and water concentrations. These results are also consistent with the fact that monomethyl sulfuric acid is a relatively poor alkylating agent.

Dimethyl Sulfate: Rate of Degradation and Formation. Dimethylsulfate is formed and consumed in a complex set of interrelated equilibria. Rates of DMS solvolysis (methanolysis and hydrolysis) can be readily measured, and these are the pathways that consume DMS. However, the amount of DMS that is formed by the forward reaction between methanol and MMS is very small. Hence, to simplify the experimental design, the DMS methanolysis and hydrolysis rates were empirically measured (eqs 4 and 5, respectively).



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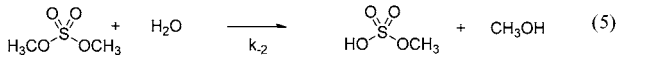

(5)

The equilibrium level of remaining dimethylsulfate following methanolysis was then used to derive the forward rate of formation for DMS.

1. Methanolysis of DMS (k_3). Solutions of 1.5 mol % DMS in methanol were heated in a sealed tube to 35 °C, and time-dependent NMR spectra were collected for these mixtures.[10] Data obtained over 60 min showed that dimethyl ether (DME) and MMS resonances gradually increased over time with a concurrent decline in the DMS signal. Representative ^{1}H NMR spectra from these experiments are presented in Figure 3. The

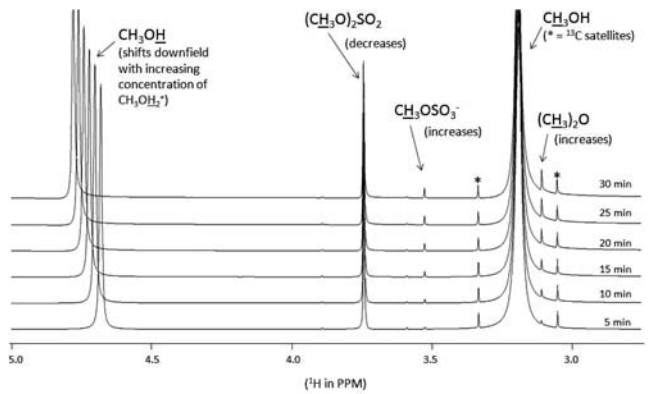

Figure 3. Time-resolved spectra for the methanolysis of DMS at 35 °C.

chemical shift of dimethyl ether was comparable to that reported in the literature and used to confirm its identity.[11] Use of NMR data helped verify the dynamic reaction pathways proposed in Scheme 1.

The gradual downfield shift of the exchangeable OH resonance (4.8 ppm) is consistent with the presence of a strong acid (i.e., MMS) as outlined in eq 4.

A duplicate set of experiments were performed under typical reaction conditions at 65 °C (again spiking 1.5 mol % DMS

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into dry methanol with <0.01% w/w H_2O and no sulfuric acid) and monitoring the reaction for 60 min. The reaction profile for the methanolysis of DMS (k_3) is presented in Figure 4. The

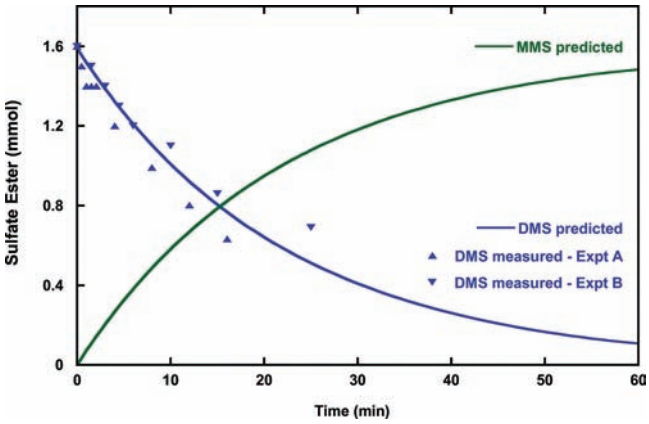

Figure 4. Experimental reaction profiles for the methanolysis of DMS at 65 °C for two replicate experiments. The DMS concentration was measured after mixing dimethylsulfate with dry methanol.

diamonds in Figure 4 represent two replicate measurements used by *DynoChem* to generate the second-order rate constant. This reaction was assumed to be essentially irreversible under nominal plant process conditions since the reaction vessels are not pressurized and the resultant DME would bubble out of solution.

The rate constant for the methanolysis of DMS (k_3) was derived from this experimental data using *DynoChem* and found to be 3.1×10^{-5} L/mol s ±10% RSD.

The rate measured by Kolesnikov and co-workers[12] for this reaction was reported as 2.27×10^{-5} s^{-1} at T = 35 °C. Kolesnikov also presented the Arrhenius parameters for this system, and they can be used to calculate the rate constant at 65 °C, which yields a value of 1.4×10^{-5} L/mol s (methanol = 23.4 M) and is comparable to the rate determined in this study.

2. Hydrolysis of DMS (k_{-2}). The hydrolysis rate of dimethyl sulfate was determined by spiking known amounts of water into a mixture of DMS and methanol at three different levels −14.3, 18.1, and 21.9 mol %. The mol % of water was calculated as [100(mol H_2O/total moles in mixture)]. The reactions were monitored for 60 min at 65 °C, and DMS peak integrals were obtained from the NMR data. Time-dependent reaction profiles for the consumption of DMS are presented in Figure 5 as the following: (a) 14.3 mol %, (b) 18.1 mol %, and (c) 21.9 mol %. The predicted DMS content (mmol) in the presence of different water spikes was performed using the k_2 (hydrolysis) and k_3 (methanolysis) rate constants.

The rate constant for the hydrolysis of DMS (k_{-2}) was derived by fitting all of the data to a *DynoChem* model that employed the time-dependent peak integral data presented above (Figure 5 a–c). The experimentally determined value for k_{-2} was found to be 1.3×10^{-4} L/mol s ±20% RSD.

Kolensikov[13] reported a value of 6.14×10^{-4} s^{-1} (T = 35 °C) for this reaction along with the Arrhenius parameters that were then used to determine the second-order rate constant at 55 °C, which was the upper temperature limit of their study. The rate constant adjusted for water concentration (H_2O = 54.8 M) was 7.86×10^{-5} L/mol s and is in good agreement with the result from this study.

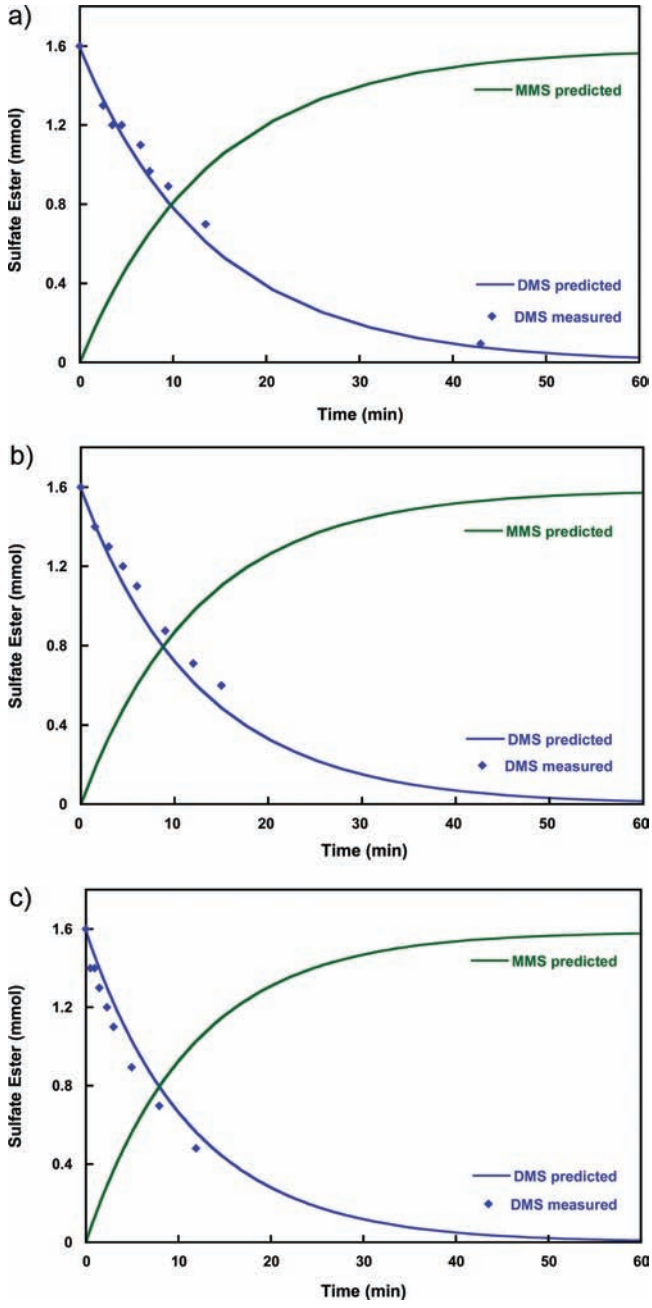

Figure 5. (a) Experimental reaction profiles for the hydrolysis of DMS at 65 °C, water spike −14.3 mol %. (b) Experimental reaction profiles for the hydrolysis of DMS at 65 °C, water spike −18.1 mol %. (c) Experimental reaction profiles for the hydrolysis of DMS at 65 °C, water spike −21.9 mol %.

Profiles of the DMS methanolysis and hydrolysis experiments revealed that MMS was formed more rapidly when the methanol solution contained water, whereas dimethylether formation was retarded by the addition of H_2O. These results suggest a bimolecular mechanism for DMS methanolysis (k_3) and hydrolysis (k_{-2}). Methanol and water compete to consume the available DMS in an S_N2-like displacement reaction. The more nucleophilic water molecule is able to hydrolyze DMS faster than dimethylsulfate can react with methanol ($k_{-2} > k_3$). However, in this reaction system, methanol is in much greater abundance and will be the primary species that consumes DMS. These two competing reactions are presented in Scheme 2.

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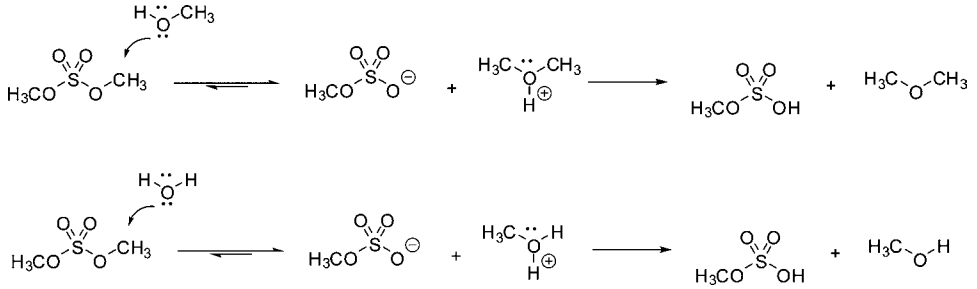
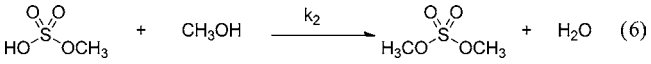
Scheme 2. Competition between methanol and water for DMS



Teasdale et al. described similar findings in their study of the methanolysis and hydrolysis of methyl methanesulfonate.[5] Their investigation using O^{18} labeled methanol confirmed that oxygen in the dimethyl ether, formed during methanolysis of methyl methanesulfonate, came from methanol and not methanesulfonic acid.

3. Formation of Dimethylsulfate (k_2). The formation of DMS from MMS can be expressed as eq 6:



(6)

Using the mechanistic pathway presented in Scheme 1, the forward reaction rate for DMS (k_2) can be calculated under steady-state conditions as follows:

(a) The rate of formation and loss of DMS are in balance

$$\frac{d[DMS]}{dt} = 0 = k_2[MMS][CH_3OH] - k_{-2}[DMS][H_2O] - k_3[DMS][CH_3OH]$$

(b) Solving for k_2

$$k_2 = \frac{[DMS](k_{-2}[H_2O] + k_3[CH_3OH])}{[MMS][CH_3OH]}$$

(c) Under dry conditions, the equation can be further simplified

$$k_2 = \frac{k_3[DMS][CH_3OH]}{[MMS][CH_3OH]} = k_3\frac{[DMS]}{[MMS]}$$

Thus, the rate constant for the formation of DMS (k_2) can be calculated if [DMS] can be measured at steady state since k_3, [MMS], and [CH_3OH] have been experimentally determined.

In early attempts to measure DMS under typical esterification conditions, the dimethyl sulfate resonance was not observed, likely because the concentration was too low (ppm) to be detected by NMR.[14] Therefore, a H_2SO_4 solution was prepared in large excess relative to the standard process conditions (0.075 mol equivalent H_2SO_4 relative to methanol or a 5-fold concentration increase) and heated for an extended time period (7 h) at 65 °C. Under these forcing conditions, a small, but clearly discernible peak was observed at a chemical shift of 3.7 ppm—the DMS resonance region. An authentic sample of DMS spiked into this mixture confirmed the peak's identity (15). The DMS peak integral area was approximately 5.7% of the upfield ^{13}C satellite peak of MMS.

The DMS peak was measured against the MMS ^{13}C satellite peak because it more closely approximated the dimethyl sulfate signal level and would better reflect the true solution concentration. The natural abundance of the ^{13}C isotope was 1.11% and equally divided between two symmetrical resonances. When corrections were applied for the number of chemically equivalent protons in DMS (6 protons) and MMS (3 protons) the steady-state DMS concentration in the reaction matrix was calculated to be 157 ppm relative to MMS,[16] resulting in a derived k_2 rate constant of 4.9×10^{-9} L/mol s.

The forward rate constant for methyl methanesulfonate formation, a closely related chemical system, has previously been reported by Teasdale[5] to be 7.1×10^{-8} s^{-1}. In that case, the rate constant was measured directly and assumed to be dependent upon conversion of a single species (methanol protonated by methanesulfonic acid) into methyl methanesulfonate and water. The rate constant was therefore expressed as a first-order process.

$$\text{forward rate} = k[CH_3SO_3^-MeOH_2^+]$$

In the current study, a second-order forward rate constant is reported since the measurement was carried out indirectly from a methanolysis reaction at equilibrium. However, a more direct comparison can be made. Since the equilibrium for the protonation of methanol by methyl methanesulfonic acid lies far to the right, the rate can also expressed as a second-order rate constant by employing the alternative convention used in this work:

$$\text{forward rate} = k_2[CH_3SO_3H][MeOH]$$

By expanding the kinetic pre-equilibration step to include the methanol concentration term (23.1 M concentration of methanol in the earlier work employing 1 M methanesulfonic acid in methanol), the first-order rate constant can be transformed into an equivalent second-order rate constant of 3.1×10^{-9} L/mol s. The DMS formation rate constant reported here (4.9×10^{-9} L/mol s) is only slightly higher than the published forward rate of methyl methanesulfonate formation.[5]

The rate constants for the entire reaction system are summarized in Table 1, as determined using the *DynoChem* software kinetics module.

Comparison of *DynoChem* Kinetic Model to Measured DMS Concentrations in a Process Stream. The derived rates constants resulted from statistical fitting of all data to a kinetic model that was generated to predict the dynamics of DMS formation/degradation. The model was subsequently used to study the formation of DMS in a simulated esterification process stream and demonstrated that steady-state levels of dimethylsulfate were formed within the typical 3-h operating window employed in the plant. Assuming anhydrous starting conditions, the steady-state concentration of DMS in the reaction

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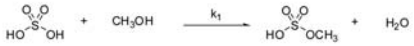
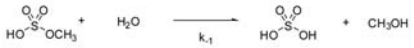
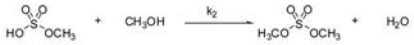
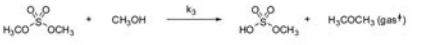
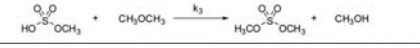
Table 1. Rate constants for the diesterification process

Step	Process	Reaction	Rate Constant (L/mole-sec)
k_1	Formation of MMS		6.5×10^{-5} ± 7% RSD
k_{-1}	Hydrolysis of MMS		$< 6.5 \times 10^{-8}$ (1)
k_2	Formation of DMS		4.9×10^{-9} (2)
k_{-2}	Hydrolysis of DMS		1.3×10^{-4} ± 20% RSD
k_3	Methanolysis of DMS		3.1×10^{-5} ± 10% RSD
k_{-3}	Formation of DMS		Reaction does not occur under conditions studied

[1]Assigned to be 999× slower than k_1 for purpose of model creation based on experimental data. [2]Confidence interval was not determined as data obtained directly from NMR experiment (not *DynoChem*) in a single measurement, value derived from experimental data collected for other rate constant.

vessel was predicted to not exceed 4 ppm in the reaction mixture.[17] Typically, reagent grade methanol used in the manufacturing plant will not be anhydrous and adventitious water would even further suppress the formation of DMS in this reaction system because the rate of hydrolysis (k_{-2}) is 5 orders of magnitude faster than formation of the DMS (k_2). However, this calculation can be used as the worst case scenario in a failure-mode evaluation of the process.

The chemical systems initially used to predict the rate constants did not contain the carboxylic acids that would be present during drug substance manufacture. Dimethyl sulfate is a strong alkylating agent and might also react with the carboxylic acid substrate, further reducing the DMS concentration in the mixture. Teasdale previously reported[5] that sulfonate ester formation was effectively quenched when the reaction mixture contained a weak base in slight excess, which was used to simulate a drug substance.

An in-process reaction mixture containing all components was tested for DMS content by GC−MS and the result was <0.5 μg/mL (ppm) as presented in Table 2, entry 1. The DMS concentration in the reaction solution was below the predicted value from the kinetic model and confirms that dimethyl sulfate was not generated at measurable levels under actual process conditions.

To further challenge the reaction system, a DMS stock solution was gravimetrically prepared and an aliquot spiked into the reaction mixture at a concentration of 1000 ppm. Samples were taken during the course of reaction and solutions analyzed for DMS using a modified GC−MS sample extraction preparation procedure. Dimethyl sulfate was present at 20 ppm after 1 h and quickly decreased to <0.5 ppm after only 3 h (Table 2).

Table 2. Analysis of DMS in reaction solutions by GC−MS using a liquid−liquid extraction procedure

entry	experimental conditions	DMS spiked in reaction mixture (μg/mL, ppm)	DMS measured in reaction mixture (μg/mL, ppm)
1	T = 0 h (analysis before DMS spike)	0	<0.5
2	T = 1 h (250× excess DMS spike)	1000	20
3	T = 3 h (250× excess DMS spike)	1000	<0.5

The API was also tested for DMS, and it was not detected in the drug substance (<0.1 ppm, the method's limit of detection).


(7)

Dimethyl sulfate that spiked into the reactor during these experiments exceeded the level predicted by the kinetic model (4 ppm in solution) by 250-fold. These results confirm that the process conditions actually control the in situ level of generated DMS. One possible explanation is the reaction between DMS and carboxylic acid substrate, which is present in large excess (3700×).[18] This reaction is illustrated as eq 7:

The effective consumption of DMS in the reaction matrix ensures that the level of this impurity does not approach the threshold for toxicological concern.

■ CONCLUSION

The formation and fate of monomethyl sulfate and dimethyl sulfate were studied by proton NMR for a methanol−sulfuric acid esterification reaction, and these results have been presented. The rate constants were determined using *DynoChem* to model the time-dependent peak integral data collected from a series of experiments probing each mechanistic step. Sulfuric acid is converted into MMS in near quantitative yield in less than 60 min under reaction conditions. Monomethyl sulfate is the more persistent of these two species and appears quite stable under process conditions. Dimethyl sulfate rapidly converts into MMS and is readily hydrolyzed by water. The presence of adventitious water further serves to reduce DMS in the reaction mixture and retards its formation. Under typical process conditions, the amount of dimethylsulfate present in the reaction solution is predicted to be less than 4 ppm. To date, DMS has not been detected in any drug substance batches (<0.1 ppm, LOD).

■ EXPERIMENTAL SECTION

NMR was chosen as the preferred method for this study, owing to its directness of measurement for all species, its wide dynamic

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range of measurement, and the fact that mechanistic assumptions could be confirmed under the same set of conditions that rate constants were being measured.

All NMR spectra were obtained in, and referenced against, DMSO-d_6 at 2.5 ppm using a Varian 500 MHz NMR. Concentrated sulfuric acid, dicarboxylic acid (starting material), and dimethyl ester (product) were obtained from the Sigma-Aldrich Chemical Co. Authentic standards of MMS (Na salt) and DMS were also obtained from Sigma-Aldrich. For spiking identification purposes, an authentic a sample of MMS was prepared by passing a methanolic solution of the commercially available sodium salt through Amberlite FPA 22 resin (H form, 10 mol equiv), and concentrating to an oil on a flash evaporator.

All small-scale reactions performed in the study were magnetically stirred and carried out using an insulated oil bath maintained at 65 ± 1 °C. Methanol used in this study contained no more than 0.01% water, and was further dried using 3 Å zeolite molecular sieves that had been predried overnight at 175 °C. Concentrated sulfuric acid, purchased from Sigma-Aldrich, was >99.9% nominal purity and was used as provided.

Sampling of reactions involved removal of a minimum of 150−400 μL of the reaction mixture and addition to DMSO-d_6 (lock solvent). The final volume in the NMR sample tube was 650 μL. Samples were chilled in an ice/water bath, and analyzed within 5−10 min of their preparation. Reaction profiles were tracked by plotting reaction completion (via reactant integral measurements) against time.

Although DMS was present in only trace amounts upon reaching equilibrium following extended methanolysis, the *S*/*N* ratio of the DMS peak was >10:1 and thus could be measured with confidence. However, MMS and its left satellite peak (nearby in chemical shift to the DMS resonance) could not be accurately integrated electronically due to the baseline deflections in this region from the broad methyl resonance of methanol (solvent). As a result, the spectrum was enlarged, and the peaks were physically extracted and weighed in order to obtain the molar ratio. The error associated with this measurement procedure was expected to be no more than 10% and comparable to other sources of experimental and computational uncertainties.

Fitting of all experimental data to generate the rate constant data and an overall kinetic model was carried out using *DynoChem* (version 3.3).

A modified GC−MS method (liquid−liquid extraction) was developed to analyse the in-process solutions and mitigate artifacts related to the sample matrix. The extraction method was also used to analyse isolated drug substance development samples. An Agilent 6890N GC was fitted with a Supelco Equity-1701 (30 m long × 0.32 mm ID, 1.0 μm film thickness) column using helium carrier gas at 2 mL/min (constant flow). Analyte detection was accomplished by an FID. The column was heated from 50 °C to 280 °C over 21 min. Approximately ∼100 mg of the sample was accurately weighed into a 10 mL conical tube, and 10.0 mL of 0.1 M NaCl was added to the sample container and vortexed for 5 min. Then, 1.0 mL of methyl *tert*-butyl ether (MBTE) was added to the mixture and vortexed for another 5 min to extract the DMS. The solution was centrifuged at 4000 rpm for 10 min, and 200 μL of the MTBE top layer was removed for analysis by GC.

DMS Spiking Experiment. A stock solution of DMS was prepared and used to challenge the disposition of dimethylsulfate under process conditions (Table 2). The final concentration of DMS in the reactor was 0.55% (5500 ppm), relative to API, and spiked into the mixture at the start of the reaction (100-g batch size). The effective DMS solution concentration was 1000 ppm. The following conditions were used for this spiking experiment:

1 Charge 100 g of dicarboxylic acid starting material.
2 Charge 580 mL of methanol and start agitation at 480 rpm.
3 Charge 21.3 g of sulfuric acid
4 Heat the reaction to 65 ± 1 °C.
5 Charge DMS, ∼0.58 g, accurately weighed (0.55 wt %/wt, or 5500 ppm, relative to the resulting API).
6 Maintain at this temperature for 3 h. Remove aliquots for analysis during 3 h reaction time.
7 Cool to 22 °C in 8 h and hold for 2 h.
8 Filter and wash cake four times with 70 mL of methanol.
9 Dry the cake at 22 °C at 100 mmHg.
10 Sample dry API for DMS content.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental data and DynoChem results from the modeling studies. This material is available free of charge via the Internet at http://pubs.acs.org

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(14) It was also possible that the weak DMS signal ($C\underline{H}_3OSO_2O\text{-}\underline{H}_3C$, 3.7 ppm) was obscured by the presence of resonances arising from the reaction products. The monoester and diester products yield ^{13}C satellite signals that reside in the same spectral region as DMS.

(15) A second minor peak was observed at 3.8 ppm and later identified as a solvent-related impurity arising from the methanol used in this study.

(16) [DMS] = (^1H peak area DMS/^1H peak area from total MMS) × (equivalent ^1H for MMS)/(equivalent ^1H for DMS) × (^{13}C abundance for MMS) = 0.057(3/6) × 0.0055 = 157 ppm.

(17) Assuming virtually anhydrous starting conditions, 2.39×10^{-5} moles of DMS will be present in the reaction mixture under steady-state conditions as predicted by *DynoChem*. The DMS amount can be normalized to the dicarboxylic starting material (1 mol) to yield a value of 24 ppm expressed as (mol DMS/mol starting material). The effective DMS solution concentration was determined to be 4 ppm by correcting for the reaction volume (~6 vols).

(18) The steady-state concentration of methyl ester intermediate was determined by *DynoChem* to be 8.97×10^{-2} mol and compared to that of DMS (2.39×10^{-5} mol) to determine molar excess (3700×).

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