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A Detailed Study of Sulfonate Ester Formation and Solvolysis Reaction Rates and Application toward Establishing Sulfonate Ester Control in Pharmaceutical Manufacturing Processes

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Abstract:

Sulfonate esters of lower alcohols possess the capacity to react with DNA and cause mutagenic events, which in turn may be cancer inducing. Consequently, the control of residues of such substances in products that may be ingested by man (in food or pharmaceuticals) is of importance to both pharmaceutical producers and to regulatory agencies. Given that a detailed study of sulfonate ester reaction dynamics (mechanism, rates, and equilibria) has not been published to date, a detailed kinetic and mechanistic study was undertaken and is reported herein as a follow-up to our earlier communication in this journal. The study definitively demonstrates that sulfonate esters cannot form even at trace level if any acid present is neutralized with even the slightest excess of base. A key conclusion from this work is that the high level of regulatory concern over the potential presence of sulfonate esters in API sulfonate salts is largely unwarranted and that sulfonate salts should not be shunned by innovator pharmaceutical firms as a potential API form. Other key findings are that (1) an extreme set of conditions are needed to promote sulfonate ester formation, requiring both sulfonic acid and alcohol to be present in high concentrations with little or no water present; (2) sulfonate ester formation rates are exclusively dependent upon concentrations of sulfonate anion and *protonated* alcohol present in solution; and (3) acids that are weaker than sulfonic acids (including phosphoric acid) are ineffective in protonating alcohol to catalyze measurable sulfonate ester even when a high concentration of sulfonate anion is present and water is absent. Implications of the mechanistic and kinetic findings are discussed under various situations where sulfonic acids and their salts are typically used in active pharmaceutical ingredient (API) processing, and kinetic models are presented that should be of value to process development scientists in designing appropriate controls in situations where risk for sulfonate ester formation does exist.

Introduction

Sulfonic acids and their derivatives have been important tools to process development chemists since they were first discovered, and they continue to be of enormous value in the manufacture of pharmaceuticals. In the pharmaceutical industry, sulfonate salts of intermediates and active pharmaceutical ingredients (APIs) are highly useful, and alcohols are frequently employed as crystallization solvents in sulfonate salt isolation processes. When a sulfonic acid and an alcohol are both present in a given process stream in any amount, there is at least a theoretical potential to form some level of an alkyl sulfonate ester impurity, regardless of circumstance. The effects of concentration, temperature, and pH may have profound impact on the real potential to form traces of a sulfonate ester impurity in any given situation, but unfortunately, there is no real information published in the chemical literature to provide guidance on the level that should be expected. In such situations analytical chemists have traditionally been required to develop assays with low limits of detection (ppm range) to determine the potential presence of sulfonate ester traces in the isolated intermediates or APIs in question. Further, when pharmaceutical candidates do transition from R&D to production, the analytical method may need to be transferred to a Quality Control lab if the established method becomes a routine specification test, in spite of the fact that the real potential for sulfonate ester formation had never been fully understood.

With the 2007 adoption of an EMEA guidance limiting genotoxic impurities to exposure limits of not more than 1.5 μg/day, the potential for sulfonate ester residues to exist in APIs has become a growing concern among regulators.[1] Simultaneously, with the publication of ICH guidelines Q8−Q10 and the pending Q11 (API Development), the pharmaceutical industry is being encouraged to adopt quality by design principles that embrace the development of predictive scientific

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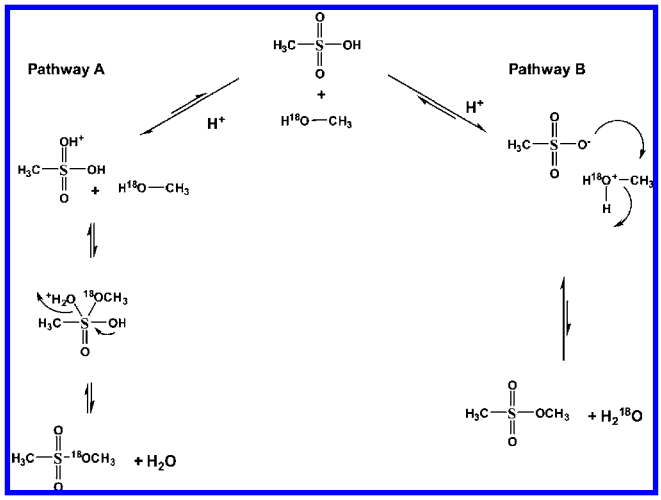

Figure 1. **Possible mechanistic pathways for sulfonate ester formation.**

knowledge to guide the design of appropriate quality controls during the development phase of each API process.

During 2007, the Product Quality Research Institute (PQRI) agreed to support a project proposal by a small group of industrial pharmaceutical development leaders to commission a detailed study of the dynamics of sulfonate ester formation and degradation. This effort was undertaken with the goals of providing mechanistic knowledge, demonstrating appropriate analytical methodology, and establishing kinetic models. Hence, with the development of these tools, the group intended not just to qualify various processing situations with respect to risk, but also to better enable industry chemists to develop appropriate control strategies when formation and carry-over risks do exist.

An initial product of this effort was a recently published communication that conclusively demonstrated the mechanism for sulfonate ester formation as being Path B of Figure 1 when methanol is reacted with methanesulfonic acid.[2] In addition, the methodologies for quantifying sulfonate esters in reaction mixtures have been developed and published.[3] In this full report, the mechanistic findings are further confirmed by kinetic studies involving systems including methanol, ethanol, and isopropanol, and representative alkyl and aryl sulfonic acids. A detailed summary of all kinetic studies conducted within the design space of time, temperature, concentration, and water content is provided.

Implications of the learning from these studies to various situations encountered by process development chemists (and of interest to regulatory agencies) are also discussed.

Experimental Section

Chemicals. Methanesulfonic acid (MSA), *p*-toluenesulfonic acid (pTSA), methanesulfonyl chloride (MSC), ethyl methanesulfonate (EMS), pentafluorothiophenol (PFTP), dimethyl sulfoxide (DMSO), ethanol (absolute, EtOH), ethanol-d_6 (EtOH-d_6), isopropanol-d_8 (iPrOH-d_8), ethanol-d_4 (MeOH-d_4), and 2,6-lutidine (ReagentPlus grade, 98%), and 2,5-dichloro-4-nitroaniline were obtained from Sigma-Aldrich (Beerse, Belgium). ^{18}O-Methanol (^{18}O-MeOH) was obtained from Isotec (Miamiburg, Ohio, U.S.A.). Pentafluoroanisole (PFA), sodium sulfate (anhydrous) and sodium hydroxide were obtained from Acros Organics (Thermo Fisher, Geel, Belgium). Methanol (MeOH) and isopropanol (iPrOH) were obtained from Biosolve (Valkenswaard, NL).

Karl Fischer Titration and Added Water Calculation. Alcohol/methanesulfonic acid mixtures used in measuring the rates of anhydrous forward reactions were confirmed to contain not more than 0.1% moisture by Karl Fischer titration, corresponding to <0.04 mol equiv relative to methanesulfonic acid inputs. In experiments where specific amounts of water were needed, actual amounts of alcohol, water, and methanesulfonic acid were measured by mass. This approach proved more consistent and accurate than using KF measurements to assess water content when appreciable amounts of water were present.

GC/MS Method for Measuring Sulfonate Ester Levels in Reaction Mixtures. *Example Internal Standard Preparation.* Methanesulfonyl chloride (1 g) was mixed with ethanol-d_6 (1 mL) in a reaction tube, closed with a Teflon lined screw cap. The reaction mixture was heated for 72 h at 70 °C. After cooling, water (2.5 mL) was added followed by diethyl ether (2.5 mL, CAUTION: volatile acidic vapors). The formed ethyl methane sulfonate-d_5 (EMS-d_5) was extracted in the ether phase. This phase was separated, dried over anhydrous sodium sulfate, concentrated under nitrogen, and diluted in acetonitrile (10 mL, CAUTION: genotoxic material). The solution was stored at 4 °C. The exact concentration of the internal standard in this solution was checked by GC/MS using liquid injection and using EMS as an external standard. The analytical conditions were similar to the conditions used for headspace analysis (see below).

Solution Preparation for Analysis. The following solutions were prepared:

- Reaction mixture: Methanesulfonic acid (or *p*-toluenesulfonic acid monohydrate) was diluted to provide a typical concentration of 100 mg/mL (around 1.04 M) in anhydrous alcohol. This reaction mixture was premixed before 1.00 mL aliquots were distributed to several 2-mL vials that were then sealed and heated concurrently at one temperature in a heating block. Water or 2,6-lutidine were added to these initial mixtures, as individual experimental needs dictated. Individual vials were sampled over time, and no vial was resampled after the seal had been initially punctured. This level of rigor proved necessary, as solvent loss in previously punctured vials otherwise was found to compromise concentration measurements.
- Derivatization solution: mixture of pentafluorothiophenol (6.4 mg/mL) and sodium hydroxide (20 mg/mL) in water.
- Internal standard solution: 100 ng/μL ethyl methanesulfonate-d_5 (synthesized) and 10 ng/μL pentafluoroanisole (system suitability test) in acetonitrile.
- Dilution solvent in SHS vials: DMSO/H_2O (1:1 by volume).

(1) *Coordination Group for Mutual Recognition-Human committee (CMDh), Request to Assess the Risk of Occurrence of Contamination with Mesilate Esters and Other Related Compounds in Pharmaceuticals*, EMEA/CMDh/ 98694/2008; European Medicines Agency: London, 27th February 2008.

(2) Teasdale, A.; Eyley, S.; Delaney, E.; Jacq, K.; Taylor-Worth, K.; Lipczynski, A.; Reif, V.; Elder, D.; Facchine, K.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *Org. Process Res. Dev.* **2009**, *13*, 429–433.

(3) Jacq, K.; Delaney, E.; Teasdale, A.; Eyley, S.; Taylor-Worth, K.; Lipczynski, A.; Reif, V.; Elder, D.; Facchine, K.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *J. Pharm. Biomed. Anal.* **2008**, *48*, 1339–1344.

- External standard solution for validation: prepared sulfonate ester standards were diluted at different concentrations between 5 and 500 μg/mL in ethanol, acetonitrile, or in reaction mixture (see above) for linearity and reproducibility tests.

GC/MS Analysis. GC/MS analyses were performed on an Agilent 6890GC-5973MSD system (Agilent Technologies, Wilmington, DE, U.S.A.), equipped with a Gerstel dual rail MPS2 sampler (Gerstel GmbH, Mülheim, Germany), according to the recently published method of Jacq et al. The available vial trays were filled as follows:

- Tray 1: 98-position temperature controlled tray for 2-mL reaction vials. The vials contained 1 mL of reaction mixture (e.g. MSA in ethanol).
- Tray 2: 32-position tray for 20-mL vials. The vials contained 2 mL of DMSO/water (1:1 mixture).
- Trays 3 and 4: two trays with 5 × 10-mL vials which contained internal standard solution, derivatisation reagent solution, and wash solvents.

The typical sample preparation sequence was as follows:

- Transfer 20 μL reaction mixture from heated tray 1 at time $t = x$ to a fresh 20 mL headspace vial (containing 2 mL 1:1 DMSO/water) in tray 2.
- Add 20 μL internal standard solution (from tray 3 or 4 to tray 2).
- Add 100 μL derivatisation solution (from tray 3 or 4 to tray 2).
- Perform headspace analysis (using headspace syringe and agitator/heater).

Between the liquid sample handling steps, syringe washing was performed using the wash solvents in trays 3 and 4.

Derivatisation completion and static headspace equilibration were achieved by maintaining the headspace vials at 105 °C for 15 min, while shaking at 600 rpm. Injection of 1 mL of headspace gas was done using a heated (110 °C) gastight syringe (2.5 mL) in split mode (1/10 split ratio) at 250 °C (split/splitless inlet temperature). Separation was performed on a 20 m × 0.18 mm i.d. × 1 μm df DB-VRX column (Agilent Technologies). Helium at 0.8 mL/min constant flow (125 kPa at 60 °C) was used as carrier gas. The oven was programmed from 60 °C (1 min) at 10 °C/min to 130 °C and at 30 °C/min to 250 °C. Detection was done by electron ionization MS in SIM mode. A solvent delay time of 3.5 min was used, and the desired ions were monitored as illustrated below for the case of analyzing for ethyl methanesulfonate:

3.0−5.5 min: 155, 183, 198 (pentafluoroanisole).

5.5−8.0 min: 79, 97, 109 (ethyl methanesulfonate), 111, 130 (ethyl methanesulfonate-d_5).

8.4−12.0 min: 200, 228 (ethyl pentafluorothiophenol, Et-TPFB), 201, 233 (Et-TPFB-d_5).

Ions 198, 109, 111, 228, and 233 were used for the integration of pentafluoroanisole, ethyl methanesulfonate, ethyl methanesulfonate-d_5, Et-TPFB, and Et-TPFB-d_5, respectively. The transfer line temperature was held at 260 °C, the source temperature at 230 °C and the quadrupole temperature at 150 °C.

(4) Elder, D. P.; Teasdale, A.; Lipczynski, A. *J. Pharm. Biomed. Anal.* **2008**, *46*, 1–8.

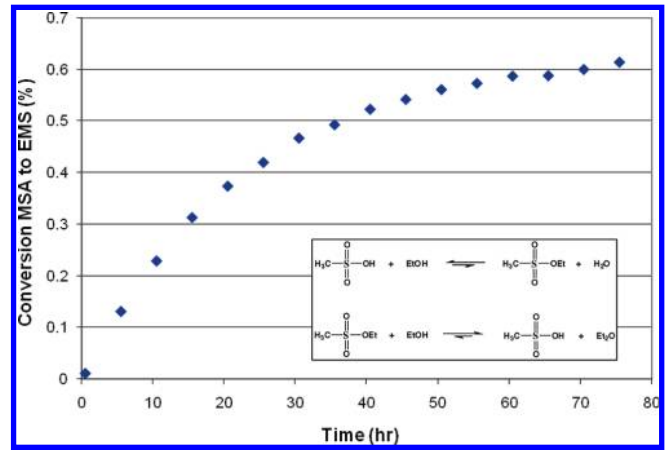

Figure 2. **Quasi-steady-state equilibrium evident for formation of ethyl methanesulfonate ester under virtually anhydrous conditions. Solution of methanesulfonic acid (1.04 M) in dry ethanol held at 70 °C.**

Utility and Limitations of the GC/MS Analysis Methodology Employed. The GC method described above and used in this work is highly sensitive and was demonstrated in this work to be capable of accurately measuring levels of sulfonate esters between 0.25 to 5 μg/mL. Use of deuterated sulfonate ester standards and the use of a ratio of area counts from alkylated thiopentafluorobenzene against those from the deuterated standards is an essential feature to achieve highly reliable results. In all studies, linearity of response was demonstrated prior to experimental measurements, and linear correlation coefficients of greater than 0.998 were shown. While the mass spectrometry detector employed would be capable of detecting still lower levels (down to 2−80 ng/mL according to the sulfonate), a practical limitation is the appearance of interfering background reactions, particularly in the case of measuring methyl sulfonate esters wherein the derivatization reagent has been shown to be alkylated by DMSO to a very small extent, even in the absence of sulfonic acid. While the method may also be used productively to measure for the presence of sulfonate ester in a specific analyte, its greatest utility in the present study was to create a window within which to detect meaningful rates of methanesulfonate formation and degradation with the precision required to allow kinetic modelling. Methodologies for API analysis have been reviewed.[4]

Kinetic Modeling Software. Rate constants and activation energies reported in this paper were obtained by statistically fitting all experimental data for each system using version 3.2 of DynoChem (Scale-up Systems Ltd., Dublin, Ireland).

Results

Studies of Sulfonate Ester Formation and Degradation under Anhydrous Conditions. Experimental work recently reported by this group has elucidated the core mechanism for methanesulfonate ester formation, and this work demonstrates that the reaction involves displacement of water from protonated methanol by methanesulfonate anion (Path B in Figure 1).[2] Under the conditions employed, the forward reaction is counterbalanced by further reaction of the methyl methanesulfonate product with the large excess of methanol present (alcoholysis) to form dimethyl ether, thereby setting up a quasi-steady-state

Table 1. **Measured kinetic and thermodynamic constants for sulfonate ester formation and solvolysis**

sulfonate ester	forward rate (s^{-1})	reference study temperature (°C)	activation energy ($kJ \cdot mol^{-1}$)	hydrolysis rate ($L \cdot mol^{-1} \cdot s^{-1}$)	alcohol	alcoholysis rate[c] ($L \cdot mol^{-1} \cdot s^{-1}$)	activation energy[d] ($kJ\ mol^{-1}$)
methyl methanesulfonate	7.10×10^{-8}	60	115[a]	3.03×10^{-6}	methanol	8.50×10^{-7}	95
ethyl methanesulfonate	7.90×10^{-8}	70	114[b]	4.80×10^{-6}	ethanol	6.00×10^{-7}	85
isopropyl methanesulphonate	2.26×10^{-7}	70	123[b]	1.09×10^{-5}	isopropanol	1.03×10^{-6}	105

[a] Forward rate constants were measured at 60, 50, and 40 °C, and the activation energy was obtained using DynoChem. [b] Forward rate constants were measured at 70, 60, 50, and 40 °C, and the activation energy was obtained using DynoChem. [c] Rate constants measured at the corresponding reference study temperature. [d] Estimate calculated from difference in equilibrium value projected at various temperatures.

equilibrium (Figure 2). In the work now being reported, this dynamic was confirmed also for systems involving methanesulfonic acid with ethanol and methanesulfonic acid with isopropanol. The forward and consumption (alcoholysis) rate constants measured at 70 °C by the GC/MS method[3] are provided in Table 1. Accordingly, this data shows that, when alcohols are combined with methanesulfonic acid at 70 °C, low but appreciable conversions to the corresponding sulfonate esters (0.3−1% recoveries) can be expected within 24 h.

In further defining the nature of the reaction, it is important to recognize that the acidity of methanesulfonic acid in dry methanol (or any other alcohol) is quite different to what one would expect when an appreciable quantity of water is present because of the suppressive effect of hydronium ion formed in competition with alcohol protonation. In the present situation the concept of the Hammett acidity function (H_0) must be applied to establish the actual extent of alcohol protonation,[5] and a published acidity function that describes the degree of protonation of ethanol in the presence of varying proportions of sulfuric acid and water provides useful context.[6] From that work, the extent of ethanol protonation as measured by NMR across the continuum of sulfuric acid and water concentrations is reproduced in Figure 3. Published H_0 (effective pH activity) values for 100% sulfuric acid (−12)[7] and for pure methanesulfonic acid (−7.9)[8] are both substantially lower than the inherent pK_a value established for anhydrous ethanol (−1.94).[5] To determine the extent of ethanol protonation by methanesulfonic acid, an NMR study was conducted. At low concentrations of ethanol in dichloromethane, several equivalents of acid appear to be required to effect full protonation of ethanol. Nonetheless, for the purpose of this study we conservatively chose to assume full protonation of ethanol as a pre-equilibrium condition in calculating all rate constants.

Although similar acidity functions for methanol and isopropanol do not appear to have been published, protonation behavior in strong acids that is very similar to that of ethanol is reported for these alcohols in the work of Weston et al.[9] which in turn allows the same conclusion to be drawn in the analogous cases of methyl and isopropyl methanesulfonate formation.

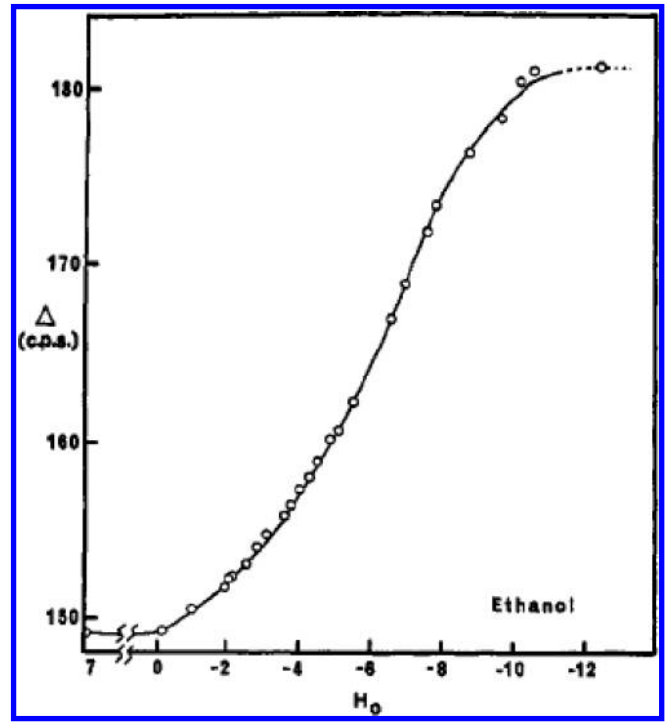

Figure 3. **Titration curve for the protonation of ethanol (reproduced with permission from ref 5). $H_0 = 0$ represents 1 M H_2SO_4 in water, and $H_0 = 12$ represents virtually anhydrous H_2SO_4 at about 18 M. The vertical axis plots the difference between NMR chemical shifts for α- and β-hydrogens.**

Given these facts and the extremely low basicity that can be expected for methanesulfonic acid itself, it is logical to assume that no more than a miniscule amount of methanesulfonic acid can possibly be protonated (first step in Figure 1, Path A) in competition with alcohol protonation (first step in Path B), even in the anhydrous state of the systems under consideration. This point likely explains the absence of any evidence for the Path A mechanism as shown in our recently published ^{18}O study.

Conceivably, the Path B sulfonate ester formation reaction could follow one of two sub paths. If methanol were to support a full separation and solvation of the ions formed (as one might expect in water), a second order kinetic relationship should be anticipated (Figure 4a). If, however, the donation of a proton to methanol results in an ion pair that is undissociated by solvation, then the forward rate would be dependent on one species only thereby making the reaction effectively first order (Figure 4b). In order to test which situation was operative the 70 °C reference reaction was repeated in each alcohol at three-quarters or one-half the starting concentration of methanesulfonic acid, and the outcome was compared against predictive models for first- and second-order reactions. In each case, the

(5) (a) Paul, M. A.; Long, F. A. *Chem. Rev.* **1957**, *57* (1), 1–45. (b) Jorgenson, M. J.; Hartter, D. R. *J. Am. Chem. Soc.* **1963**, *85* (7), 878–883.
(6) Lee, D. G.; Cameron, R. *J. Am. Chem. Soc.* **1971**, *93* (19), 4724–4728.
(7) Farcasiu, D.; Ghenciu, A. *Prog. Nucl. Magn. Spectrosc.* **1969**, *29*, 129–168.
(8) Paul, R. C.; Kapila, V. P.; Kumar, R.; Sharma, S. K. *J. Inorg. Nucl. Chem.* **1981**, *43* (1), 171–172.
(9) Weston, R. E.; Ehrenson, S.; Heinzinger, K. *J. Am. Chem. Soc.* **1967**, *89* (3), 481–486.

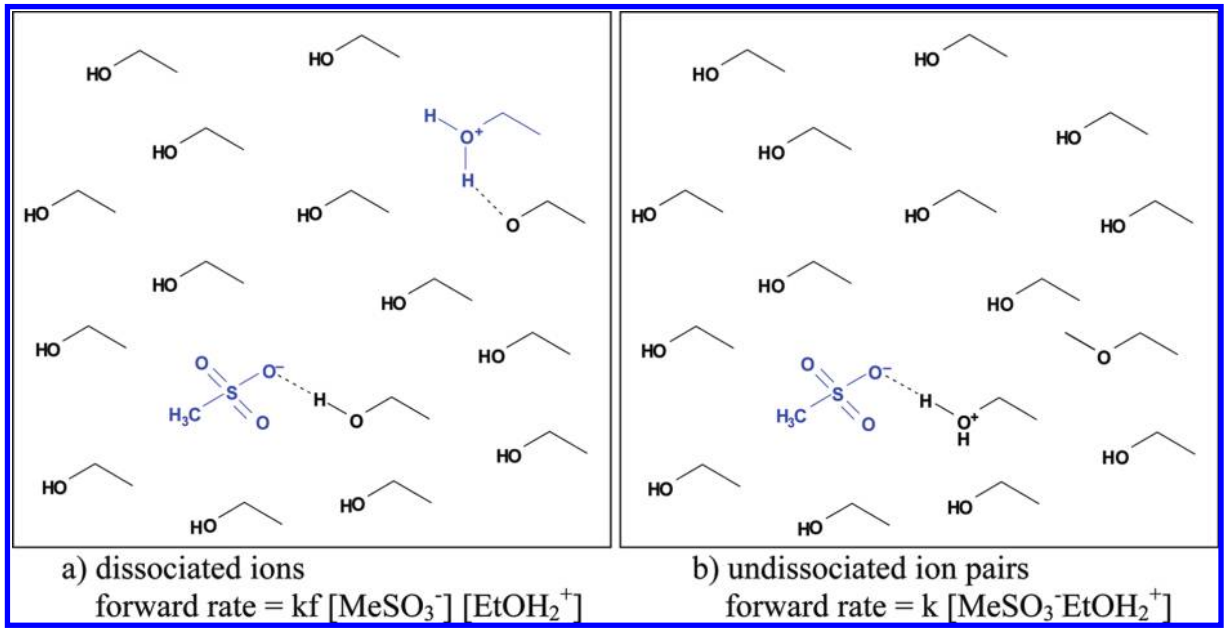

Figure 4. **Mechanistic possibilities for sulfonate ester formation.**

outcome overlapped much more closely with the prediction for a first-order reaction (as exemplified in Figure 5), thereby indicating that the anhydrous forward reaction occurs almost exclusively between closely associated methanesulfonate anion and protonated alcohol ion pairs that have acquired sufficient energy to effect displacement of water from the latter by the former.

Effect of Temperature on Sulfonate Ester Formation and Degradation under Anhydrous Conditions. While reference forward reactions were measured at 70 °C for 1 M methanesulfonic acid in both anhydrous ethanol and isopropanol, rate constants were also obtained at 40 °C, 50 °C, 60 °C, and 70 °C allowing for the measurement of activation energies for each scenario, as also shown in Table 1. The activation energy for the forward rate in the case of 1 M methanesulfonic acid in dry methanol was similarly measured, except that the 70 °C data was not obtained owing to the lower boiling point of methanol (65 °C). The availability of these data allows predictive models to be used to estimate the rate of sulfonate ester formed at any temperature and time when essentially anhydrous conditions are present (see discussion section, below). A representative graph showing rates of isopropyl methanesulfonate formation starting from 1 M methanesulfonic acid in dry isopropanol at various temperatures is provided in Figure 6.

Effect of Added Base on Sulfonate Ester Formation and Degradation under Anhydrous Conditions. The GC/MS method was employed to determine rates of methanesulfonate ester formation starting from methanesulfonic acid and alcohols (ethanol and isopropanol at 70 °C, and methanol at 60 °C) in the presence of slight molar excesses or deficiencies of an added weak base, 2,6-lutidine, as a mimic for a basic API. In each case, no measurable rate of sulfonate ester was observed when even a slight excess of base was employed. When a 2% excess of sulfonic acid was present, however, a very slow rate of sulfonate ester was observed as illustrated in Figure 7 (0.004% conversion after 12 h illustrated for the EMS case). This is consistent with what should be expected from a highly diminished level of free sulfonic acid relative to the 1 M concentration described at 70 °C in Figure 2. Meanwhile, the lack of measurable rate when an excess of 2,6-lutidine is present would indicate that the conjugate acid of 2,6-lutidine ($pK_a \approx 4$) is not sufficiently strong as an acid to enable a meaningful degree of alcohol protonation (consistent with conclusions of the prior work cited on ethanol basicity[8]). To test this point further, experiments involving a slight excess of 2,6-lutidine over methanesulfonic acid were remeasured in the three alcohols at high temperature, but with a 10% overage of concentrated phosphoric acid added to overwhelm the 2,6-lutidine excess. Again, no measurable rate of sulfonate ester was detected in any of the three experiments. Finally, as a positive control this experiment was repeated using a slight excess of 2,5-dichloroaniline over methanesulfonic acid, thereby generating a 1 M concentration of the conjugate acid of the former (pK_a of −1.78 on the H_0 scale[5b]). In this case, a significant, but predictably diminished rate of sulfonate ester was evident (Figure 7) owing to the reduced capability of the 2,5-dichloroanilinium ion to effect alcohol protonation relative to methanesulfonic acid.

From a qualitative standpoint, this set of experiments demonstrates that a good correlation exists between alcohol protonation and acid (or conjugate acid) strength. Most importantly, it demonstrates that even under anhydrous conditions, an acid strength exceeding that of phosphoric acid is required to effect meaningful alcohol protonation in order to promote even a slow rate of sulfonate ester formation. Based on these findings it seems reasonable to apply the learning from our previous labeling experiments to other alcohols.

Impact of Alcohol, Water, Acid, and Salt on Sulfonate Ester Degradation. The formation of sulfonate ester rises as a sulfonic acid reacts with excess alcohol, but as buildup occurs alcoholysis becomes competitive, reducing the net rate of formation, resulting ultimately in a steady state concentration of the sulfonate ester. Rate constants for alcoholysis of methyl, ethyl, and isopropyl methanesulfonate were measured at 70 °C, and are reported in Table 1. Together with forward rate constants, these data allow for the accurate prediction of

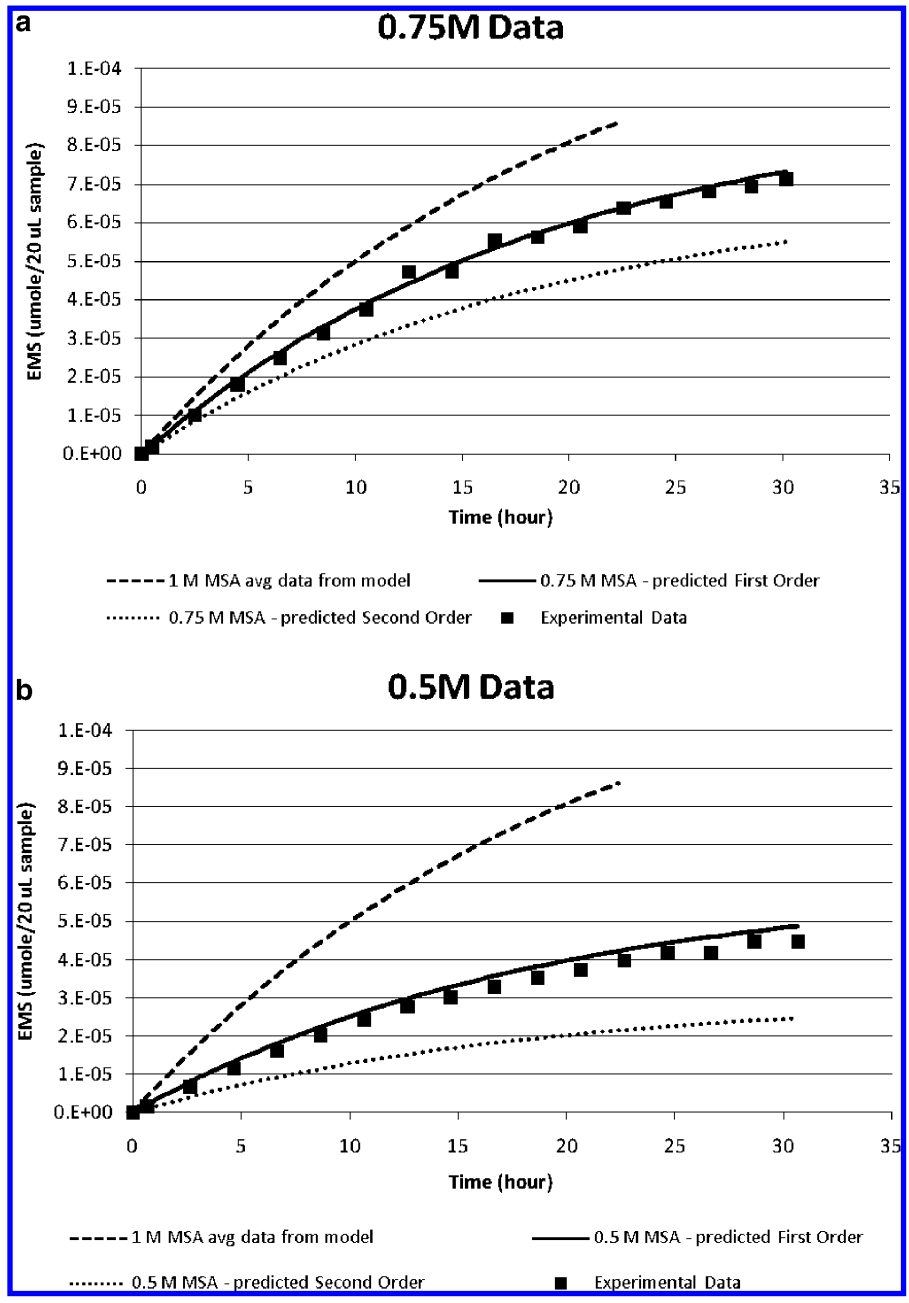

Figure 5. **Predicted outcomes for kinetic profiles related to first- and second-order reactions and experimental results.**

sulfonate ester generation as a function of temperature. Essentially identical rate constants were observed in each case when a small amount of either methanesulfonic acid or 2,6-lutidine is included when reaction between each sulfonate ester and alcohol was measured. For example, pseudo-first-order observed rate constants for solvolysis of EMS at 70 °C were 1.44×10^{-5} s^{-1} in ethanol and 1.45×10^{-5} s^{-1} and 1.36×10^{-5} s^{-1} in the presence of lutidine and MSA, respectively. Hence, the alcoholysis rates were not subject to a meaningful level of either acid or base catalysis.

The addition of water to the anhydrous condition reduces initial rates considerably owing to competition of water for protons that would otherwise associate with alcohol molecules. Sulfonate ester buildup is also suppressed by a second, faster solvolytic pathway for degradation (hydrolysis). Rate constants for hydrolysis were measured as reported in Table 1 for methyl, ethyl, and isopropyl methanesulfonate, and these rate constants were similarly found to be unaffected by deviations from pH neutrality. As would be expected, the presence of water dramatically reduces both the steady-state equilibrium value of sulfonate ester that can be achieved relative to the anhydrous case, and it also reduces the rate at which that equilibrium is achieved (see example in Figure 8). In fact, in order to allow meaningful levels of sulfonate ester to be measured with the cited assay, it is necessary to increase the amount of methanesulfonic acid when high ratios of water to alcohol (e.g., 8:1) are employed (Figure 9).

It would seem likely that the first-order behavior observed for sulfonate ester formation under anhydrous conditions would not hold fast when substantial amounts of water are added. Hydration should be expected to result in extensive dissociation of the ion pairs, resulting in a shift toward pure second-order behavior as described in Figure 4b. In order to test this possibility, we conducted reactions with varying proportions

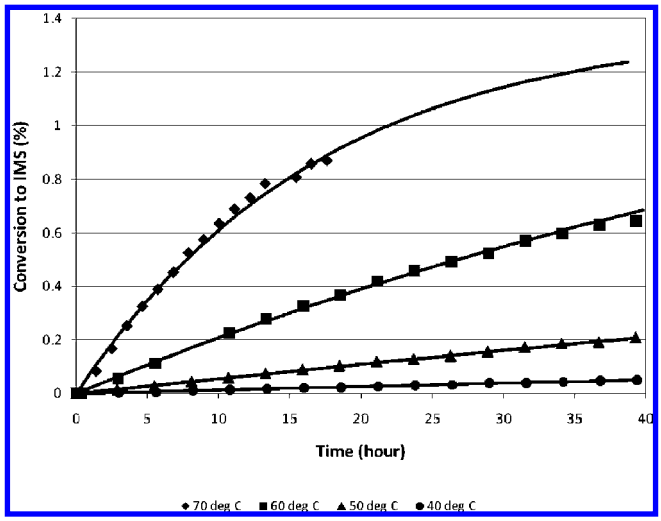

Figure 6. **Rate of isopropyl methanesulfonate ester formation in anhydrous isopropanol as a function of temperature. Solid lines represent data predicted by fitted DynoChem model.**

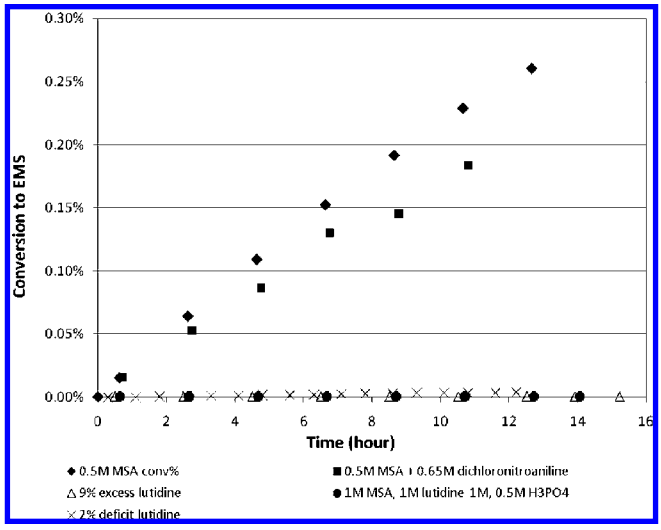

Figure 7. **Rate of ethyl methanesulfonate ester formation at 70 °C in the presence of various scenarios of added base.**

of alcohol and water, and found that at best there is a mixture of first- and second-order behavior, with the latter being increased as the proportion of water is increased. One might expect that the addition of a salt (e.g., sodium perchlorate) would help break up ion pairs, resulting in greater second-order behavior, but experimentally we found a slight tendency toward greater first-order behavior in this case. Thus, while it would seem ideal to be able to model sulfonate ester formation under any condition of water/alcohol mixture, this goal proved too difficult to achieve within the scope of the current work. Nonetheless, the point can be made that the presence of water has a dramatic effect in reducing sulfonate ester levels, and even if pure first order reaction were to be conservatively assumed, the quasi-equilibrium level of ethyl methanesulfonate illustratively decreased by approximately 5-fold when 5% water is included at 70 °C, and correspondingly by about 1,500-fold (0.0004% conversion after 15 h) when the content of water was increased to 67% relative to ethanol (Figure 8).

(10) Gerber, C.; Toelle, H.-G. *Toxicol. Lett.* **2009**, *1903*, 248–253.

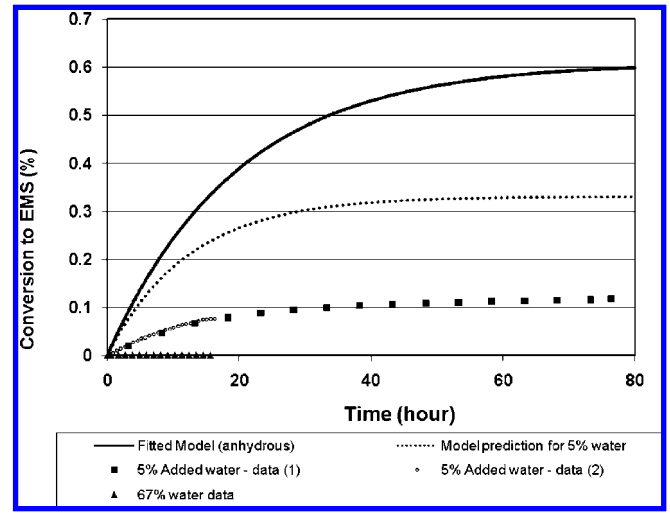

Figure 8. **Effect of added water on the formation of EMS from MSA (1 M in aqueous ethanol) at 70 °C. The observed correspondence when overlaying the two sets data for '5% added water' demonstrates the intermediate precision of the methodologies used.**

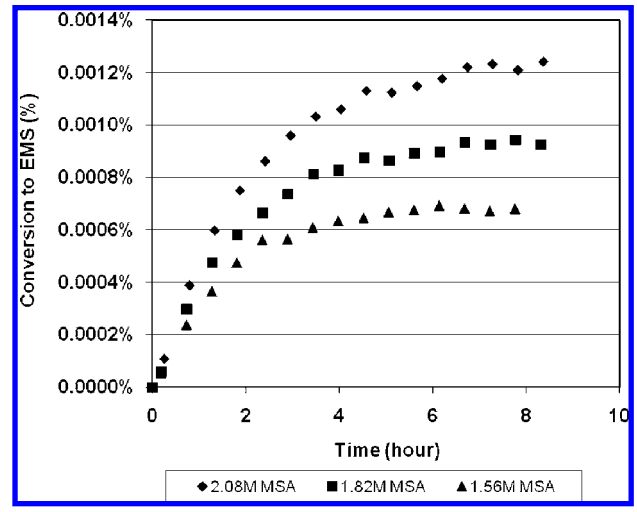

Figure 9. **Extent of conversion to EMS and equilibrium reached when various concentrations of MSA are heated to 70 °C in 12 mol % ethanol/88 mol % water.**

Demonstration of Analogous Behavior by *p*-Toluenesulfonic acid. Tosic (*p*-toluenesulfonic) acid is another commonly employed member of the sulfonic acids. It is typically sold and used commercially as its monohydrate form. Consequently, kinetic studies on the anhydrous form would be meaningless to carry out since the material would not be typically employed in an anhydrous state. To demonstrate that sulfonate ester formation rates in the presence of alcohol are relatively close to those of methanesulfonic acid, a comparative rate study was performed in the presence of similar levels of water. The results (shown in Figure 10) show similar rates indicating general consistency of reaction behavior.

Discussion

The intended goal of this study was to provide both industrial chemists and pharmaceutical regulators with a more detailed understanding of the conditions under which sulfonate esters may be formed and/or degraded, and to demonstrate the application of a recently published analytical technique by which

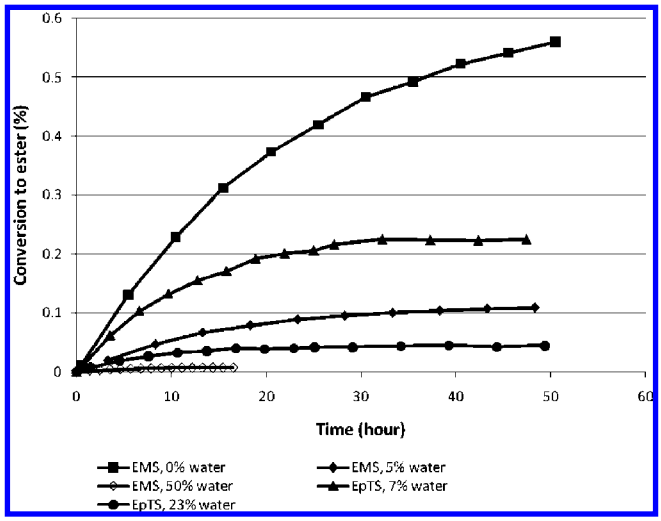

Figure 10. **Impact of water on ethyl methanesulfonate and ethyl *p*-toluenesulfonate formation (7 mol % water corresponds to use of $TsOH \cdot H_2O$ to prepare 1 M solution).**

even slow rates of sulfonate ester formation may be measured. Importantly, the results show that sulfonate ester formation is of greatest concern under conditions where anhydrous alcohol comes into contact with a sulfonic acid under conditions of high temperature, or alternatively at lower temperature but under highly prolonged contact.

The measured rate constants and activation energies provided in Table 1 can be readily used to predict the amount of sulfonate ester that can form under a variety of conditions. It has been shown that the mechanism of sulfonate ester formation requires that any alcohol present must be appreciably protonated for reaction to occur, and that such protonation can only begin to occur when the alcohol is exposed to highly acidic/anhydrous conditions.

Hence, the most effective way to avoid even a trace rate of sulfonate ester formation when making an API sulfonate salt is to employ an excess of the API as base. In cases where this tactic may be highly disadvantageous, the degree of sulfonate ester formation may be readily calculated at a given temperature using, for example, the simulation tool in Dynochem in conjunction with the Arrhenius activation energy data provided in this paper. For example, if 2,6-lutidine were to represent an API of 1 M concentration in anhydrous ethanol and a 2% molar excess of methanesulfonic acid were to be employed in a crystallization procedure at 15 °C to effect salt formation, the DynoChem simulator would apply rate constant information derived from the measured activation energies for ethyl methanesulfonate formation and alcoholysis to determine that 2×10^{-5} molar concentration of ethyl methanesulfonate (950 ppm relative to 2,6-lutidine) could form once the steady-state equilibrium is reached (Figure 11 (left)). However, it also calculates that it would require about 4.5 years in order to reach that state! Within the more typical processing time frame of a 12 h overnight hold period at 15 °C, the simulation predicts that only 3×10^{-8} molar concentration of ethyl methanesulfonate (Figure 11 (right)) would actually form (about 1.6 ppm on a molar basis relative to that of the 2,6-lutidine present), and this of course does not take into account the purging of ethyl methanesulfonate to the mother liquor that would be

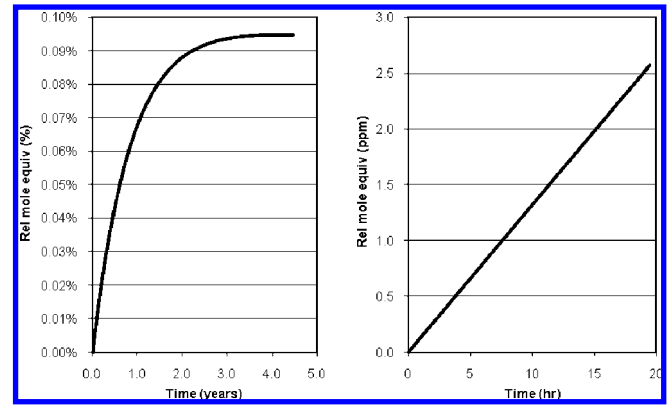

Figure 11. **Illustrative application of kinetic model - see text for scenario details.**

expected when the API sulfonate salt is isolated following crystallization.

At the other extreme, if a solution of methanesulfonic acid in anhydrous ethanol at 1 M concentration were allowed to reach steady-state concentrations resulting in the formation of about 0.3 M% yield of EMS through a combination of temperature and time and an API (salt or otherwise) were allowed to contact such a solution without the benefit of a careful filtration and wash, then a considerable amount of ethyl methanesulfonate could be readily measured in the API. Such an event, unfortunately, occurred in a highly publicized incident during 2007 involving the HIV drug Viracept, but this represented an extreme and highly unusual case.[10] Using the kinetic modeling program DynoChem, outcome predictions are provided in Table 2 from a number of much more typical processing scenarios.

These data demonstrate that in the majority of cases the formation of sulfonate esters is not a significant issue, even when placed into the context of the 1.5 μg/day threshold of toxicological concern (TTC) standard. Moreover, in the event that the predicted level of sulfonate ester approaches such toxicologically based limits, it would seem that solubility and purge studies, using the analytical methodology presented in this paper, could be used on a one-time basis to demonstrate that a high level of control is present.

Thus, a key conclusion from this work is that the high level of regulatory concern over the potential presence of sulfonate esters in API sulfonate salts is largely unwarranted and that sulfonate salts should not be shunned by innovator pharmaceutical firms as a potential API form.

The following key understandings are provided for process chemists who are considering forming an API sulfonate salt in the presence of an alcohol to minimize sulfonate ester formation to the lowest practical level:

(1) Use an exact stoichiometry or an excess of the API base in order to completely eliminate the potential for sulfonate ester formation.
(2) If an excess of sulfonic acid is needed, use the minimum excess possible and conduct the salt formation and isolation steps at the lowest practical temperature.
(3) Include water in the salt formation and isolation procedures to competitively minimize the concentration of protonated alcohol and to take advantage of the strong hydrolysis rates relative to rates of ester formation.

Table 2. **Demonstration scenarios where models may be applied**

	processing scenario	alcohol concentration (M)	free sulphonic acid concentration (M) excess over base	temperature (°C)	maximum time of exposure (h)	maximum concentration of sulfonate ester in solution (ppm relative to API)
1	API sulphonate salt is formed with 5% molar excess MSA in ethyl acetate, but solvent contains 100 ppm ethanol	0.002	0.005	60	15	2.5
2	API sulfonate salt is formed with 2% molar excess MSA in acetone containing 5% isopropanol to improve crystallisation	0.83	0.0125	5	20	0.6
3	API freee base is present in 2% excess relative to MSA, and salt is crystallized from pure alcohol	21.7	0	78	3	$\ll 1$
4	API free base is reacted with 10% excess MSA to form salt in THF containing 20% methanol (at moderate temperature)	6.24	0.025	40	8	159

(4) Avoid situations in which dry sulfonic acid and anhydrous alcohol are mixed and stored before use.

Conclusions

Pharmaceutical companies have employed sulfonic acid salts of APIs for many years, and often such salts have proven to be the form of choice for a given API. Throughout recent years innovator companies that produce (sulfonate salts in the presence of alcohols) have been required to provide evidence that sulfonate esters are not present in the APIs above a very low threshold any time a sulfonate anion is involved and the process for its formation had involved an alcohol in any concentration and at any pH.

The work reported in this paper was undertaken to fill a void of scientific information to allow both industrial chemists and regulators to approach this issue more scientifically.

The general conclusions from this work are that:

(1) Appreciable levels of sulfonate esters only form under highly acidic/anhydrous conditions in conjunction with elevated temperature. Even under these forcing conditions, <1% conversion takes place.

(2) Traces of alcohol carried over from a prior step do not pose a problem if the intended solvent for API salt formation is nonalcoholic (e.g., THF). Even when a pure sulfonic acid is allowed to interact with an alcohol, temperature and reactant concentrations are the determinants of whether meaningful levels of sulfonate ester can form. The extent of formation can be predicted under a wide range of circumstances from the Arrhenius activation energy data presented in this paper.

(3) Reaction between a sulfonic acid and an alcohol follows a first-order kinetic pathway that indicates that reaction occurs almost exclusively between ion-pairs of protonated alcohol and sulfonate anion. When water is added to such a system, there is a dramatic reduction in forward rate owing to the competing protonation of water rather than alcohol, an increased proportion of second-order reaction, and a 10-fold higher rate of sulfonate ester hydrolysis over the inherent rate of sulfonate ester formation. Even when high temperature is employed in a process, the inclusion of water can play an important suppressive role to sulfonate ester formation.

(4) Increasing the proportion of water to alcohol leads to a transition from first order (internal ion-pair displacement) to second order (ion separation by hydration leading to classic S_2N displacement) kinetics, making it impractical to create a single general model allowing complete prediction encompassing all potential combinations of process parameters. However, it is questionable as to whether a more detailed model would ever be required since the use of the kinetic models under anhydrous conditions as provided in this paper can provide adequate enough information regarding any process scenario involving water while generally embedding a high level of conservatism in the underlying assumptions.

In a broader sense, the work described in this paper illustrates that alcoholic solvents need not be avoided in processes leading to API sulfonate salts if appropriate processing conditions are chosen. Furthermore through the application of the simple processing rules described, control over ester formation can be so effective as to also potentially eliminate the need to routinely test for esters in isolated API.

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Supporting Information Available

This information is available free of charge via the Internet at http://pubs.acs.org.

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