Short communication

Detection of low-level PTFE contamination: An application of solid-state NMR to structure elucidation in the pharmaceutical industry

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ABSTRACT

We report a novel use of solid-state ^{19}F nuclear magnetic resonance to detect and quantify polytetrafluoroethylene contamination from laboratory equipment, which due to low quantity (up to 1% w/w) and insolubility remained undetected by standard analytical techniques. Solid-state ^{19}F NMR is shown to be highly sensitive to such fluoropolymers (detection limit 0.02% w/w), and is demonstrated as a useful analytical tool for structure elucidation of unknown solid materials.

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1. Introduction

Polytetrafluoroethylene (PTFE) is widely employed in coatings for laboratory equipment and pharmaceutical manufacturing instruments, due to its desirable chemico-physical properties: high operating temperature (260 °C), low coefficient of friction, good abrasion and chemical resistance [1]. In a recent crystallisation slurry experiment of a developmental fluorinated active pharmaceutical ingredient (API), ^{19}F solid-state nuclear magnetic resonance (SSNMR) data surprisingly showed an unexpected resonance. In some cases, the relative integral compared to that of the API was >20%, but remained undetected by other analytical techniques including HPLC, NMR, IR, Raman and infrared spectroscopy. The impurity was subsequently identified by SSNMR as PTFE contamination from equipment used during the slurrying process.

In this communication we demonstrate the usefulness of SSNMR for solving problems encountered in pharmaceutical solids. SSNMR is becoming an increasingly important tool for studying polymorphism of drugs [2,3], which was in fact the original aim of this work. At GlaxoSmithKline, SSNMR has continuously expanded from studies of polymorphs and solvates of APIs [4] to more advanced studies

of organic co-crystals and complexes [5,6] and determination of crystal structure from powder samples [7]. In the following discussion we show that SSNMR evidence can be valuable for structure elucidation of unknown insoluble materials.

2. Experimental

2.1. Crystallisation slurry

Competitive slurry experiments were performed on mixtures of two polymorphs, called form A and form B, of a developmental API. 100 mg of each form were slurried in 1.5 mL of acetone, isooctane or a mixture of ethyl acetate and acetone (64:36) at temperatures between −20 and 60 °C, for 2 h to 63 days. The slurry experiments were performed in 2 mL glass screw cap vials, stirred at approximately 500 rpm using a PTFE coated magnetic stirrer bar in a Microvate reaction rack (RAR-12). The samples were isolated into a Bond Elut® filtration tube (polypropylene tube and polyethylene frit) using nitrogen pressure and dried under vacuum at 40 °C for *ca.* 20 h. A total of 165 samples were isolated.

2.2. HPLC

Reverse phase gradient HPLC analysis using a polar embedded column with a water/methanol mixed mobile phase buffered with ammonium hydrogen carbonate was performed on an Agilent 1100 system with UV detection.

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2.3. FTIR

Fourier-transform infrared (FTIR) spectroscopy was performed on a PerkinElmer Universal ATR Spectrum One infrared spectrometer using diamond attenuated total reflectance (ATR) sampling at $2\,cm^{-1}$ resolution for 16 scans (ca. 2 min scan time).

2.4. Solution-state NMR

Solution-state NMR spectroscopy was performed using a Bruker Avance 400 NMR spectrometer (^{1}H and ^{19}F frequencies of 400 and 377 MHz, respectively), equipped with a Bruker 5 mm BBFO autotune probe. ^{1}H and ^{19}F spectra, with and without proton decoupling, data were generated on samples of API with concentrations of ca. 10 mg/mL in DMSO-d_6. ^{1}H and ^{19}F chemical shifts were referenced to tetramethylsilane (TMS) and CFCl$_3$ respectively. The temperature was regulated to 300 K during data acquisition.

2.5. Solid-state NMR

^{13}C and ^{19}F SSNMR spectroscopy experiments were performed on a Bruker Avance 360 wide-bore solid-state NMR spectrometer (^{1}H, ^{13}C and ^{19}F frequencies of 360, 90 and 339 MHz, respectively), equipped with a Bruker 4 mm magic-angle spinning (MAS) HFX probe. Approximately 70 mg of sample was packed into a zirconia 4 mm rotor and spun at 8 kHz for acquisition of ^{13}C data, and at 14 kHz for acquisition of ^{19}F data. The temperature was regulated at 295 K. Direct polarisation (subsequently referred to as ^{13}C MAS and ^{19}F MAS) and cross-polarisation [8,9] (subsequently referred to as ^{13}C CP MAS and ^{19}F CP MAS) methods with ^{1}H decoupling employed during free precession were used. The ^{1}H nutation frequency of the decoupling pulse was 100 kHz; TPPM [10] and SPINAL-64 [11] decoupling schemes were used for ^{13}C and ^{19}F data acquisition, respectively. For the cross-polarisation experiments, contact times of 3 and 1 ms were applied for ^{13}C CP MAS and ^{19}F CP MAS, respectively. During the contact time, the nutation frequencies of ^{1}H, ^{13}C and ^{19}F were ca. 60 kHz; the amplitude of the ^{1}H square pulse was ramped down to 50% at the end of the pulse. Quantitative analysis used the ^{19}F MAS method with 64 scans co-added. To ensure fully quantitative data, the recycle delay was set to 10 s, which is at least 5 times the ^{19}F spin-lattice relaxation times, T_1(F), of the expected components of the samples—^{19}F T_1s of two polymorphs of the API were measured as ca. 2 s by using saturation recovery [12], T_1(F) of PTFE was estimated <2 s at room temperature [13,14]. ^{13}C and ^{19}F chemical shifts were referenced to TMS and CFCl$_3$, respectively.

3. Results and discussion

3.1. Structure elucidation of the unknown material

The original objective of the crystallisation slurry, performed on 1:1 mixtures of forms A and B of the API, was to study the relative stabilities and kinetics of a solvent-mediated polymorphic transformation [15]. The API molecule contains two types of fluorine nuclei, aliphatic and aryl. Fig. 1a shows an expanded region of a ^{19}F MAS spectrum of a typical slurry sample. The aliphatic fluorine resonates at −63 ppm; at a 14 kHz MAS rate, its first spinning sideband appears at −104 ppm. The aryl fluorine resonates in the −115 to −119 ppm region, where high resolution allows for discrimination of forms A and B and their two crystallographically independent molecules ($Z' = 2$).

In 89 of the 165 slurry samples, an unknown ^{19}F species, resonating at an isotropic chemical shift of −121.3 ppm (Fig. 1a), was detected at variable levels, 0.5–21.2% integral ratio relative to the aryl fluorine of the API. It should be noted that, initially, the presence of this resonance, which also coincides with the aryl region of
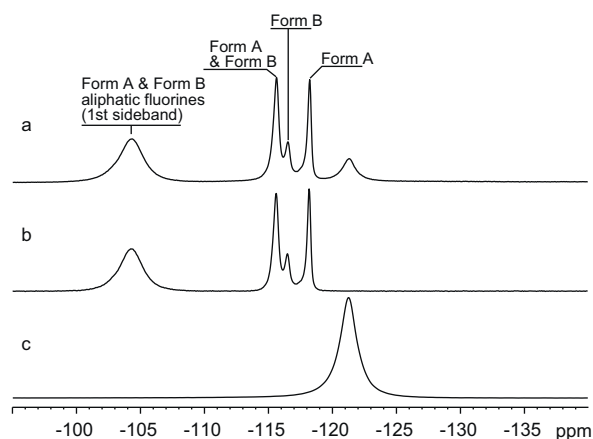

Fig. 1. Expanded spectral region: (a) ^{19}F MAS and (b) ^{19}F CP MAS of API polymorph mixture after an 11-day slurry in ethyl acetate at 60 °C; (c) ^{19}F MAS of PTFE, obtained as Klingerflon commercial thread seal tape. The integral of the −121.3 ppm signal in the ^{19}F MAS was calculated as 21.2% relative to the integral of the API.

the two forms of the API, raised concerns about the existence of an additional polymorph, since slurry experiments have been known to create new polymorphs [16]. A second, more comprehensive slurry programme was devised to include other analytical techniques, including HPLC impurity profile analysis, NMR, IR, Raman and infrared microscopy. None of these techniques could detect any additional species. HPLC analysis indicated that the impurity profile and levels of impurities were consistent throughout the study and typical for batches of this compound. Vibrational spectroscopy and microscopy results were inconclusive. Solution-state ^{19}F NMR did not indicate any resonances other than that of the API. At this stage, the evidence suggested an additional polymorph of the API.

Proton spin-lattice relaxation times, T_1(H), are generally distinguishable between different polymorphs of an organic compound [2,17]. However, when attempting the measurement of T_1(H) via ^{19}F cross-polarisation (CP), we discovered that the CP step resulted in complete loss of signal for the unknown species. Fig. 1b shows a ^{19}F CP MAS spectrum of the same sample as in Fig. 1a. The missing signal at −121.3 ppm indicated that the unknown material did not contain any hydrogen nuclei, providing evidence for the presence of PTFE. Loss of the CP signal due to rapid molecular motions can be disregarded in this case since the aryl fluorine of the API can be considered rigid regardless of crystal forms. Fig. 1c shows a ^{19}F MAS spectrum of PTFE reference material, where the isotropic chemical shift of the CF$_2$ group can be seen at the same position, −121.3 ppm, and is in good agreement with values reported in the literature (ca. −120 ppm [18], −122 ppm [19]).

Furthermore, ^{13}C CP MAS yielded no signals other than that of the API, while ^{13}C MAS gave a broadened resonance at 111.1 ppm, which is not one of the chemical shifts of the API (data not shown) and is consistent with the reported chemical shift of PTFE (111.3 ppm [20]). A ^{13}C resonance broadened by ^{19}F–^{13}C dipolar coupling would be expected for PTFE since ^{19}F heteronuclear decoupling was not employed during free precession.

To summarise, SSNMR evidence identified PTFE as the unknown material. It is present in more than half the number of slurry samples, detectable at level >0.5% integral ratio relative to the API. We concluded that the contaminant must have originated from the coating of the stirrer bar as the only possible source of the polymer. Finally, a slurry sample showing the highest integral ratio of PTFE by SSNMR was dissolved and filtered through a gold leaf. An IR spectrum of the solid remainder was concordant with a PTFE reference spectrum.

It is observed that, due to the overwhelmingly large number of CF$_2$ units, the two CF$_3$ terminals have an insignificant effect on
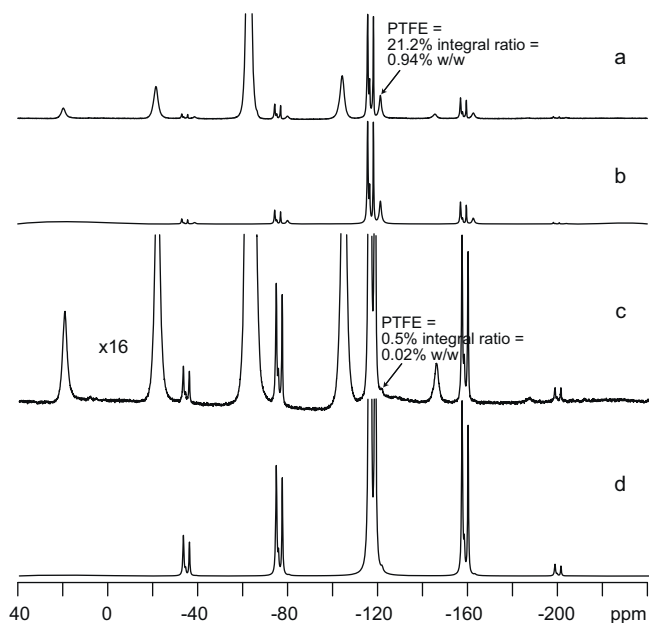
Fig. 2. (a) and (c) are experimental ^{19}F MAS spectra of slurry samples containing 0.94 and 0.02% w/w PTFE, respectively. In the same order, (b) and (d) are their deconvolution model spectra. The vertical scale of the 0.02% w/w PTFE spectrum was expanded by 16×. The following five spinning sideband patterns were deconvolved: API aliphatic fluorine at well-resolved isotropic chemical shift of −62.8 ppm; aryl fluorine (API) at three isotropic chemical shifts of −115.6, −118.2 and −116.5 ppm; and PTFE at isotropic chemical shift of −121.3 ppm. See text for further detail.

the chemical shift of the CF_2. Consequently, PTFE of any molecular weight must resonate at a single isotropic chemical shift of *ca.* −121 ppm; the detection of PTFE at this chemical shift is independent PTFE molecular weight.

3.2. Quantitative analysis of PTFE

Since the NMR experimental condition of full relaxation was satisfied, the integral of the ^{19}F MAS signal could be used to obtain the level of PTFE in weight percentage units without the requirement for a gravimetric calibration curve. However since the peaks of interest were not completely resolved, the spectrum was deconvoluted in order to obtain more accurate integrals than possible with routine integration. We used the DMfit program [21] for this task. Model CSA (chemical shift anisotropy) MAS lineshapes were fitted into the experimental spinning sideband patterns of the ^{19}F MAS spectrum. For demonstration purposes, Fig. 2a shows an experimental ^{19}F MAS spectrum of a slurry sample. The model CSA MAS lineshapes were calculated from the sideband patterns. The model spectrum, which is the sum of all lineshapes, is shown in Fig. 2b. For clarity, the aliphatic fluorine lineshape had been removed as the PTFE weight percentage was quantified by comparison to the integral of the aryl fluorine of the API only. The DMfit integration procedure accounted for all sidebands in a lineshape.

For the aromatic fluorine in the API, ^{19}F homonuclear dipolar coupling was estimated to be <1 kHz from single-crystal structure, and was considered completely averaged out by MAS (14 kHz). Deconvolution therefore accurately yielded both the integral and the CSA of the API, though we do not make use of the latter. The situation is not the same for PTFE, where the ^{19}F homonuclear dipolar coupling between the rigid CF_2 spin pairs is large, *ca.* 15 kHz [22,23], and can only be completely averaged out at MAS rate >18 kHz [22].
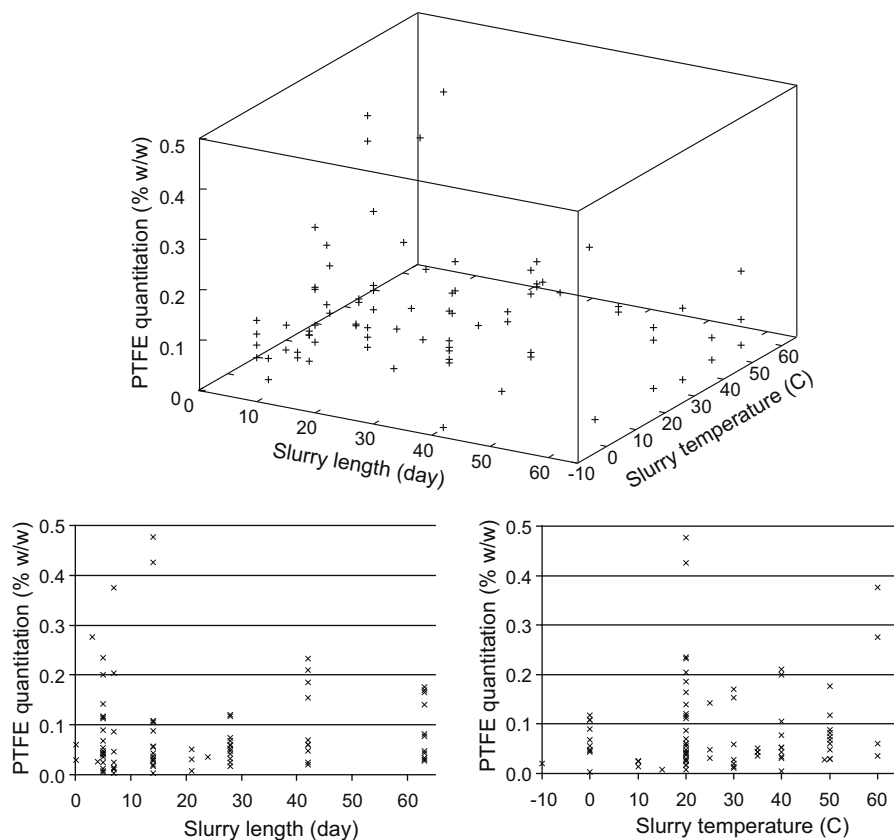

Fig. 3. Top: plot of PTFE contamination (% w/w) against slurry length and temperature in 89 of 165 slurry API samples (some samples containing 0.5–1.0% w/w PTFE are not shown). Bottom: projections on the length and temperature axes.

The CSA yielded by the deconvolution of a spectrum at 14 kHz MAS rate contains a certain error due to the contribution from the dipolar coupling. This, however, did not seem to affect the goodness of fit since the magnitude of the residual spectrum was very small, 0.3% relative to the experimental spectrum of PTFE. The integral obtained from the deconvolution of the PTFE spectrum can therefore be considered accurate.

The molecular weight of a polymer can be practically considered as a multiple of the molecular weight of its repeating monomer unit, M_{mono}, without making any significant error. By using simple algebraic manipulations, one can derive the following equation for calculating the weight percentage of a polymer in a small-molecule API sample:

$$c = \frac{100}{(z_{mono}/z_1)(I_1/I_2)(M_1/M_{mono}) + 1} \tag{1}$$

where c is the weight percentage (% w/w) of the polymer; I_1, z_1, and M_1 are the NMR integral, the number of nuclei contributing to the integral, and the molecular weight of the API, respectively; I_2 and z_{mono} are the NMR integral of the polymer and the number of nuclei in the monomer, respectively. Note that according to Eq. (1), the weight percentage of the polymer is independent of its molecular weight. This underlines the advantage of the NMR method for quantification of polymers, as accurate molecular weights of polymers are often not straightforward to determine. Quantification using Eq. (1) has also been crosschecked by a single-point gravimetric analysis. 119.64 mg of a slurry sample, which showed 0.94% w/w PTFE by ^{19}F SSNMR, was dissolved in acetone; the solution was filtered, and the residue had a mass of 1.35 mg, which equates to 1.1% w/w, in good agreement with the NMR quantitation. Since NMR analysis is inherently quantitative, a calibration curve is not required and gravimetric analysis on other slurry samples was not pursued.

Consider a system of a typical small molecule like our API having a molecular weight of 712.73, and a fluoropolymer such as PTFE. Since ^{19}F is a 100% natural abundance nucleus, a sufficient ^{19}F signal-to-noise ratio for a minimum level of the fluoropolymer, for instance 0.5% integral ratio, should be achievable within minutes of run time. Substituting this integral ratio to Eq. (1), and using $M_{mono} = 100.02$ for the molecular weight of PTFE monomer unit (C_2F_4), $z_{mono} = 4$ and $z_1 = 1$, the weight percentage of PTFE is calculated as 0.02% w/w, or 25-fold less than the integral ratio. This explains the high sensitivity of ^{19}F SSNMR method to fluoropolymers. Fig. 2c demonstrates a limit of detection of 0.02% w/w achievable for PTFE in slurry samples.

Fig. 3 summarises the results of the quantitative analysis of PTFE using the SSNMR method on a range of slurry samples. There appears to be very little correlation between either slurry length and temperature to the amount of PTFE present. We have concluded that the shedding of PTFE was more likely the result of friction between the coating and slurried solid particles or glass vessel rather than chemical erosion. Note that PTFE was evident in two samples prepared under fairly mild conditions, namely up to 4 h at 20 and 60 °C; PTFE level 0.04 and 0.08% w/w, respectively. Moreover, the contamination was found in samples made independently in two different laboratories. The evidence therefore suggests that the problems may be ubiquitous when magnetic bars are used for stirring. Though PTFE is not considered to be of any significant health risk, such a high level of contamination may create more serious risks of erroneous quantitative analysis of other quality-critical impurities in the API.

4. Conclusions

We have reported that under fairly mild conditions, PTFE coatings can be abraded from the surface of laboratory equipment and contaminate the sample at levels up to 1% w/w. This finding has not yet been reported in the literature. The quantification of PTFE was shown to be independent of the molecular weight of the polymer, underlining the benefit of the NMR method since an accurate molecular weight of polymer is often difficult to obtain. ^{19}F SSNMR was shown to have excellent sensitivity to fluoropolymers such as PTFE (with a detection limit 0.02% w/w), with the advantage that data can be generated on solid powder samples 'as is'. We envisage SSNMR to be a useful analytical tool for structure elucidation and quantification of similar types of fluoropolymers, for instance polymerised fluorinated degradation products.

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