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Analytical approaches to investigate salt disproportionation in tablet matrices by Raman spectroscopy and Raman mapping

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ABSTRACT

It has always been challenging to use spectroscopic methods to analyze salt disproportionation in a multi-component tablet matrix due to the spectral interference generated by the various excipients. Although combining Raman spectroscopy and chemometrics can be a powerful approach to study the extent of salt disproportionation, it was found in the present study that bulk measurements and chemometric modeling have obvious limitations when the targeted component is present at low levels in the tablet. Hence, a two-step Raman mapping approach was developed herein to investigate salt disproportionation in tablets with a low drug loading (5% w/w). The first step is to locate the area of interest where the drug particles reside throughout the tablet surface by using a statistically optimized sampling method termed deliberate sub-sampling. The second step, referred to herein as close-step mapping, utilize a step by step mapping of the targeted area to find more details of salt disproportionation in the tablet regions where the drug is concentrated. By using this two-step Raman mapping approach, we successfully detected the existence of minor species embedded in multi-component low drug loading tablet matrices, where bulk measurements from routine techniques usually lack of sensitivity. This approach will help formulation scientists detect and understand salt disproportionation and *in situ* drug-excipients compatibility issues in low dose solid dosage formulations.

1. Introduction

Most drug substances are reported to be either weak acids or weak bases [1]. Formulating poorly water-soluble weak acids or weak bases into salts can not only increase bioavailability, but also improve many other physicochemical properties [2–4]. Hence, salt formation of active pharmaceutical ingredients (APIs) is recognized as one of the most widely used formulation approaches [5]. However, a potential risk of employing salt formation is that salt forms of APIs have a propensity to revert back to the unionized form/free base, a process termed salt disproportionation [6,7]. Disproportionation, a proton exchange involving an acid base reaction, can be significantly influenced by several factors including the physicochemical properties of excipients, drug loading, and storage conditions [8]. Theoretically, the tendency for disproportionation can be anticipated based on assessment of the microenvironment and knowledge of the salt $pH_{max,}$ based on the assumption that disproportionation is a solution mediated process [5,9]. It has been reported that salt disproportionation has detrimental effects on pharmaceutical product performance including loss of potency, increased tablet hardness, and reduced dissolution rate and bioavailability [10–14]. Therefore, it is essential to develop a mechanistic understanding of how excipients lead to disproportionation and, perhaps more importantly, develop more sensitive approaches to detect disproportionation.

A variety of analytical methods have been employed to reveal influential factors in disproportionation. Raman spectroscopy was shown to be a useful tool to quantify the free base and to study effects of excipients on the integrity of salts of weak bases in a binary mixture [6]. For example, coupling multivariate analysis with vibrational spectroscopy, Christensen et al. demonstrated that acidic excipients can induce the calcium salt of atorvastatin to disproportionate at elevated temperature and humidity [15]. John et al. illustrated that the proton accepting and hygroscopic

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capacity of excipients have significant impact on salt disproportion in powder blends [16]. However, there are limited studies of salt disproportionation in tablet matrices containing commonly used components due to the analytical interference from excipients as well as challenges with limits of detection (LOD) when the drug is present at a low weight percent, as is common in tablet formulations. Therefore, development of an applicable analytical method with an improved LOD is highly desirable to detect the existence of low-level unwanted species in multi-component systems.

Taking advantage of higher Raman scattering coefficients of active pharmaceutical ingredients (APIs) compared to those of typical excipients, Raman mapping is capable of detecting low levels of APIs and related substances by generating a chemical map with high lateral resolution [17–21]. For instance, Widjaja et al. successfully targeted magnesium stearate, a minor component in a model pharmaceutical tablet, by applying Raman microscopy and multivariate analysis [22]. Šašić demonstrated the spatial distribution of alprazolam in commercial tablets with API contents lower than 1% (w/w) by combining Raman mapping with chemometrics [23]. It has been reported that a minor amount of a polymorph, as low as 0.025% w/w, in a low-content API formulation was detectable by an innovative method which combined Raman mapping with statistically optimized sampling [24,25]. Applying a similar method to salt disproportionation studies might enable quantitation of low levels of free base embedded in a multi-component formulation.

The primary objective of this study is to develop applicable methodologies for conventional Raman mapping to detect minor components resulting from disproportionation and to understand the "*in-situ* compatibility issues" between drug and excipients in a pharmaceutical tablet with low drug loading. In addition, by using chemometrics and Raman spectroscopy, we are expecting to further explore the impact of commonly used excipients and storage conditions on the kinetics of salt disproportionation in tablets. Pioglitazone (PIO) hydrochloride (HCl) salt, a compound with high efficacy and tolerability to treat type II diabetes, was selected as a model compound for disproportionation studies, based on previous reports of the existence of free base in the bulk powder of the salt and the low calculated pH_{max} value [16,26–28]. In this study, we used chemometric modeling and bulk Raman measurement of 30% drug loading (DL) tablets to reveal the kinetics of disproportionation for PIO-HCl at different storage conditions. We also applied a statistical sampling method (deliberate sub-sampling) to identify the suspected disproportionation area on the surface of 5% DL tablet. After identification of regions of interest (drug particles dispersed in the tablet matrix), a close-step mapping approach was conducted on the area of interest to further investigate the disproportionation of PIO-HCl in a low content API tablet. By using the proposed methods, we could successfully discern the minor component in a multicomponent matrix, which was difficult to achieve by using bulk Raman measurements and chemometrics.

2. Experimental

2.1. Chemicals

Pioglitazone hydrochloride salt was purchased from TCI Co., Ltd. (Portland, OR). Pioglitazone neutral forms/free base were obtained from Dr. Reddy's Laboratory (India). Magnesium Stearate (Mgst) was obtained from Mallinckrodt (St. Louis, MO). Sodium Croscarmellose (CCNa) and Avicel PH-101 were purchased from FMC Biopolymers (Philadelphia, PA). Crospovidone was supplied by ISP Tec. (International Specialty Products, Wayne, NJ). Mannitol was ordered from SPI Pharm. (Wilmington, DE). Anhydrous Dibasic Calcium Phosphate (A-TAB) was purchased from JRS Inc. (Patterson, NY). Chemical structures of API and excipients used in this study are shown in the supporting information (Fig. 1SA–D).

2.2. Tablets preparation

Formulations of calibration tablets and the tablets for stability tests are listed in the supporting documents (Tables 1S and 2S). Powders of API and excipients were gravimetrically dispensed (+/− 2% weight standard deviation) into a 20 ml glass vial by a Symyx Powdernium powder dispensing robot. The weighed powders were mixed by a LABRAM mixer (Butte, MT) with 30% intensity for 3 min. 100 mg of the mixture was carefully poured into a 7.14 mm diameter tablet die held in place with a flat-faced lower punch. Afterwards, the flat-faced upper punch was gently placed on the powder bed. Axial pressure (200 MPa) was applied to the powder bed by a single station MTS Alliance RT equipped with a 50 kN (variation +/− 1N) loading cell (MTS Systems Corporation, Eden Prairie, MN). Tablets ($n = 6$) formulated with Mgst, CCNa, or A-TAB were subjected to stability studies at 40 °C/75% R.H. (relative humidity) or 40 °C/35% R.H. chambers in an open dish condition.

2.3. Raman spectroscopy bulk measurement

Raman spectroscopy bulk measurements were conducted by employing a 785 nm Kaiser Raman RXN1 Workstation (Kaiser Optical Systems, Inc., Ann Arbor, MI) equipped with a PhAT™ large volumetric bulk sampling probe utilizing HoloGrams™ v4.1 software for data acquiation. The PhAT probe was connected to the base unit *via* a fiber bundle (1 fiber excitation, 50 fiber collections) to deliver the excitation energy from the Invictus™ diode laser located within the base unit. Laser power was set electronically to 400 mW for the bulk measurement. A 250 mm focal length was employed to generate a 6 mm laser spot. Utilizing both the Kaiser Hololab™ calibration accessory and a Raman calibration standard, calibrations of the spectrograph wavelength (neon atomic lines; ASTM standard), laser excitation wavelength (cyclohexane; ASTM standard) and instrument spectral response (white-light source; NIST standard) were performed to ensure spectral quality. Taking into account the penetration of the PhAT Raman measurement, powder samples (each component of tablets) were placed into a beaker with controlled thickness (20 mm) for reproducibility. The tablets were placed on specially designed tablet holder which could be affixed to the stage of the workstation. To mitigate the baseline offset due to dark noise from the camera, the CCD was cooled to −40 °C and a dark subtraction was automatically applied to each spectrum. Data acquisition time for Raman spectroscopic bulk measurements was set as 10 s and 6 accumulations were averaged for all the samples. All Raman spectral data was processed by Grams AI (Thermo, Inc., Waltham, MA) for visual inspection. Multivariate analysis software SIMCA v13 (Umetrics AB, Umeå, Sweden) was used for chemometric analyses.

2.4. Raman microscopy

Kaiser Raman RXN1 workstation, along with both Kaiser HoloMap and HoloGrams software packages, were used for Raman mapping experiments. To avoid sample damage, the Invictus laser power was set to 50% of the maximum (200 mW). A single mode excitation fiber, a multimode collection fiber (100 μm), and a 50× microscope objective were used to increase spatial resolution of Raman imaging. The size of the laser spot on the surface of sample was measured before the experiments by direct visualization and measurement under the optical microscope with the laser powered up. The visual laser spot size was found to range from 10 to 15 μm on the tablet surface. Samples were placed on a tablet holder affixed to a motorized stage (Prior Scientific, Fulbourn, United Kingdom).