Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 852–859

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Using Raman spectroscopy in tablet moisture surface analysis: Tablet surface markers

Eman Atef[a,*], Harsh Chauhan[a], Michelle Ceresia[b], Charles Pidgeon[c,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, Boston, Massachusetts College of Pharmacy and Health Sciences, 179 Longwood Avenue, Boston, MA 02115, United States
[b] Department of Pharmacy Practice, School of Pharmacy, Boston, Massachusetts College of Pharmacy and Health Sciences, 179 Longwood Avenue, Boston, MA 02115, United States
[c] Rombult Professor of Pharmaceutical Sciences and Intellectual Property Officer, School of Pharmacy Boston, Massachusetts College of Pharmacy and Health Sciences, 179 Longwood Avenue, Boston, MA 02115, United States

ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 12 June 2010
Accepted 14 June 2010
Available online 19 June 2010

Keywords:
Raman spectroscopy
Tablet surface markers
Lactose
Mannitol
Loratadine
Surface bound moisture
Indirect moisture measurement

ABSTRACT

A method was developed to monitor the hydration of a tablet surface using chemical functional groups able to bind atmospheric water through H-bonding. In this study, generic oral dissolving loratadine tablets were used. These tablets have relatively high mannitol and lactose concentrations. Both mannitol and lactose have C–OH alcohol functional groups, several of which are potentially available for H-bonding with atmospheric water. The Raman intensity of the alcohol functional groups decreases upon hydration. This observation can be used to indirectly monitor water adsorbed to tablet surfaces at the alcohol sites. The hydration assay is based on the change in the Raman peak intensity of the alcohol C–OH stretching at 875.5 cm^{-1}. Consequently the decrease in the Raman intensity of this vibration can be used to monitor water adsorption. The Raman measurement of tablet surface water was compared to the direct moisture measurement method using a microbalance. The Raman spectroscopy is used to monitor the water that is specifically bound to the C–OH alcohol functional groups available for hydration. The microbalance was used to monitor the tablets' weight change during water adsorption and desorption. The distribution of the ratio of the Raman intensity of C–OH peak at 875.5 cm^{-1} divided by the intensity of loratadine's C–Cl peak at 712.6 cm^{-1} was experimentally determined to be a Gaussian distribution with a mean of 3.22 ± 0.277. Raman analysis indicates that there is both tightly and loosely bound water at the tablet surface. This can be a useful technique with regard to inspecting and controlling the tablet drying process.

1. Introduction

It is self-evident that tablet manufacturing produces chemical functional groups (CFGs) on the dosage form surfaces that are proportional to the compounds used. However, if the active pharmaceutical ingredient (API) is present in a small concentration in the tablet, the API CFGs will not dominate the tablet surface. When excipients constitute the major tablet ingredients, then the excipients' CFGs can significantly influence the tablet surface chemistry and hydration properties, particularly for uncoated tablets. In our study Walvert loratadine 10 mg, generic orally dissolving tablets (ODTs) were used.

Raman spectroscopy can analyze (i) micron to millimeter depths within tablets (ii) or can completely penetrate the tablet during transmission spectroscopy measurements. Thus, depending on both how the Raman laser excitation beam is focused and the power of the laser beam, the excitation laser beam can penetrate only a small distance into the surface or through the entire tablet [1,2]. We used Raman spectroscopy as a technique to measure water specifically bound to the ODT excipients alcohol functional groups. Raman spectroscopy has been used for (i) chemical fingerprinting of solids, (ii) determination of hydration [3,4] and (iii) analysis of crystallinity and crystal polymorphism [5]. Quantitative analysis using Raman Spectroscopy has increased over the past few years [6–9]. Moreover the use of Raman spectroscopy in tablets and powders mapping has increased as demonstrated by several industrial applications [10–13].

ODTs are designed and manufactured to release the API in the oral cavity. ODTs contain soluble excipients to facilitate dissolution and drug release from the dosage form in the oral cavity. They are also prepared using techniques that facilitate quick water uptake and disintegration.

Different polymorphic forms are used in ODTs; however, the amorphous forms are more susceptible to hydration than crystalline polymorphs [14], and consequently are the polymorph of choice when preparing ODTs [15]. Most ODT excipients are hygro-

Abbreviations: CFG, chemical functional groups; PAT, process analytical techniques; TSMs, tablet surface markers; ODTs, oral dissolving tablets.

* Corresponding authors.
E-mail address: Chuck.pidgeon@mcphs.edu (C. Pidgeon).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.06.009

scopic and may deliquesce under high humidity conditions [16]. The two predominant excipients in the Walvert tablets are mannitol and lactose. Both exist mainly in a crystalline polymorphic form. Mannitol is a common excipient in ODTs due to its negative heat of solution and sweetness.

Lactose and mannitol have 8 and 6 alcohol CFGs, respectively. The Raman bands for the C–OH stretching frequency at 875.5 cm^{-1} decreases in intensity when the alcohol groups are hydrated. The available hydration sensitive C–OH CFGs can be used to monitor the adsorbed water at these sites on the tablet surface. Nevertheless, in general, monitoring Raman active bands that change intensity when hydrated demonstrates that CFGs may be utilized as markers (tablet surface markers or TSMs) for characterizing tablet properties. For uncoated tablets exposed to different humidity and temperature conditions, TSMs can monitor an existing or changing surface property, e.g. surface hydration as shown in this work. Tablet surface CFGs that are Raman active are referred to as TSMs throughout this study. For example, mannitol or lactose molecules themselves are not the TSMs, but rather the TSMs are the alcohol CFGs associated with these excipients.

Oral cavity saliva liquids are not in the gas phase. Therefore, tablet hydration in the oral cavity occurs by a different mechanism, solution state hydration, compared to the vapor phase hydration performed in our study. The common polymorphic forms of lactose are: (i) α lactose, (ii) β lactose, and (iii) amorphous lactose. The α lactose polymorph exists as either anhydrous or monohydrate form, both are available as monophasic. Commercially available β lactose is a polymorphic mixture comprising approximately 20–30% α lactose [17]. Mannitol is a polymorphic excipient commonly used as a β form. Other forms like alpha, delta and hydrates may exist as contaminants [18].

Atmospheric moisture interacting with pharmaceutical solids in either bulk form or in tablet form can cause (i) water surface adsorption, (ii) water absorption into bulk solids, which can cause deliquescence [19], (iii) crystal hydration formation and (iv) for solids with microvoid spaces, capillary condensation occurs within the microvoids even at low relative humidity. The process of water condensation in microvoids can result in occlusion of the microvoid channels [20]. Regarding process (ii), when bulk lactose is exposed to the atmosphere, a hydrate–anhydrate phase transformation can occur [21]. The surface of pharmaceutical tablets are also susceptible to these interactions when tablets are exposed to atmospheric moisture, but frequently the surface chemistry of a particular tablet formulated and manufactured under a specific condition can modify the physical chemistry of the dosage form surface. Virtually all Raman active bands obtained from mannitol and lactose bulk powders are also observed in the Raman spectra of intact tablets prepared from these excipients. This enables the use of Raman spectroscopy to analyze tablets comprised of these two excipients. As previously indicated our focus is the CFGs that can be used as reporter-chemical groups elucidating a surface property, in this case hydration, rather than the actual excipient.

2. Material and methods

Bulk powder loratadine (L9664), lactose monohydrate (L3643) and β lactose (L3750) were purchased from Sigma–Aldrich (St. Louis, MO). Mannitol USP donated from Specialties (Wilmington, DE). Walvert tablets were purchased from a local Walgreens Pharmacy. Blister Pack Walvert tablets are the loratadine brand equivalent to Alavert®. Lyophilized amorphous lactose was prepared using a 5% lactose aqueous solution [22]. The sample was frozen at −80 °C then lyophilized using a Labconco Freezone 4.5 Console model for 24 h. The Raman spectra of 48 tablets, prior to hydration, were used to analyze the frequency distribution function of surface accessible C–OH CFGs. Frequency distribution functions are sometimes referred to as a Probability distribution functions and abbreviated as PDF.

2.1. Tablet composition

In this study commercially available Walvert tablets were used. The low concentration tablet components are the following: artificial cherry flavor, aspartame, colloidal silicon dioxide, sodium lauryl sulfate and sodium stearate. We evaluated other Walvert excipients' Raman active peaks. These excipients included; povidone, microcrystalline cellulose, lactose monohydrate and mannitol. Our goal was to find out if some of the label excipients exist in high concentration in the Walvert tablets and whether they have interfering peaks with the TSMs C–OH peak. Their Raman spectra were evaluated as follows.

The absorbance at 1656 cm^{-1} ($\bar{\nu}$ C=O), the most distinct povidone peak in the Raman spectrum was studied [23,24]. This was determined by subtracting the loratadine spectrum from the Walvert tablet surface spectrum. The 712.6 cm^{-1} C–Cl loratadine peak was used as a reference for subtraction.

The label also indicates that the tablets have microcrystalline cellulose. We studied the 1095 cm^{-1} and 1292 cm^{-1} the two major peaks in the Raman spectrum of microcrystalline cellulose [25].

As indicated by the manufacturer, the tablets contain lactose monohydrate. However there appears to be a small amount of amorphous lactose contaminating the lactose monohydrate. USP-NF states that various proportions of amorphous lactose may exist in lactose monohydrate [26]. Lactose monohydrate and mannitol were identified as major excipients in the tablet using their characteristic Raman peaks (Fig. 1).

The ratio of lactose to mannitol in the tablet was experimentally evaluated. Raman spectroscopy was used to measure the lactose monohydrate to mannitol weight ratio in experimentally prepared solid state mixtures. Lactose monohydrate and mannitol were mixed together in a mortar using a pestle for 1 min at four different ratios 1:9, 1:3, 1:1 and 3:1. The Raman spectrum of each mixture was obtained and used to generate a calibration curve. The peak intensity ratio of the $I\bar{\nu}$ 934 cm^{-1} (specific to mannitol)/$I\bar{\nu}$ 851 cm^{-1} (specific to lactose), versus the mannitol percentage weight was plotted. Regression analysis of the log-plot elicited a straight line; the resulting calibration equation was $y = 42.424E^{-4.885x}$ where $y = I\bar{\nu}\,934\,cm^{-1}/I\bar{\nu}\,851\,cm^{-1}$ and x is the mannitol percentage weight. The calibration curve was done in triplicate.

2.2. Measurement of tablet surface hydration and polymorphic changes

The humidity chamber of a 500 mL desiccator jar was equilibrated with 300 mL of distilled water at 50 °C in a THELCO Oven (Model-17) for 3 h. Hydration of blister pack Walvert tablets involved (i) opening the blister pack, (ii) obtaining a Raman spectra prior to hydration, and then incubating the test tablets in the humidity chamber for 180 min (3 h).

When tablets were removed from the incubator a continuous Raman spectrum was measured for the hydrated tablets for 90 min using irradiated target areas of (~80–100 μm) diameter on the tablet surface. This method enabled monitoring the intensity of the moisture sensitive alcohol peak at 875.5 cm^{-1} for changes in peak intensity due to water adsorption of the tablet surface. To monitor the polymorphic conversions of lactose and mannitol during the adsorption desorption process, 10 tablets were stored under the same conditions. Polymorphic changes were monitored using the α lactose peaks at $\bar{\nu}$ 851.0 cm^{-1} and $\bar{\nu}$ 1086 cm^{-1} and the β lactose peak at $\bar{\nu}$ 895 cm^{-1} and, whereas the β and δ mannitol were monitored using the 1037 cm^{-1} and 1052 cm^{-1} peaks, respectively (Fig. 1).

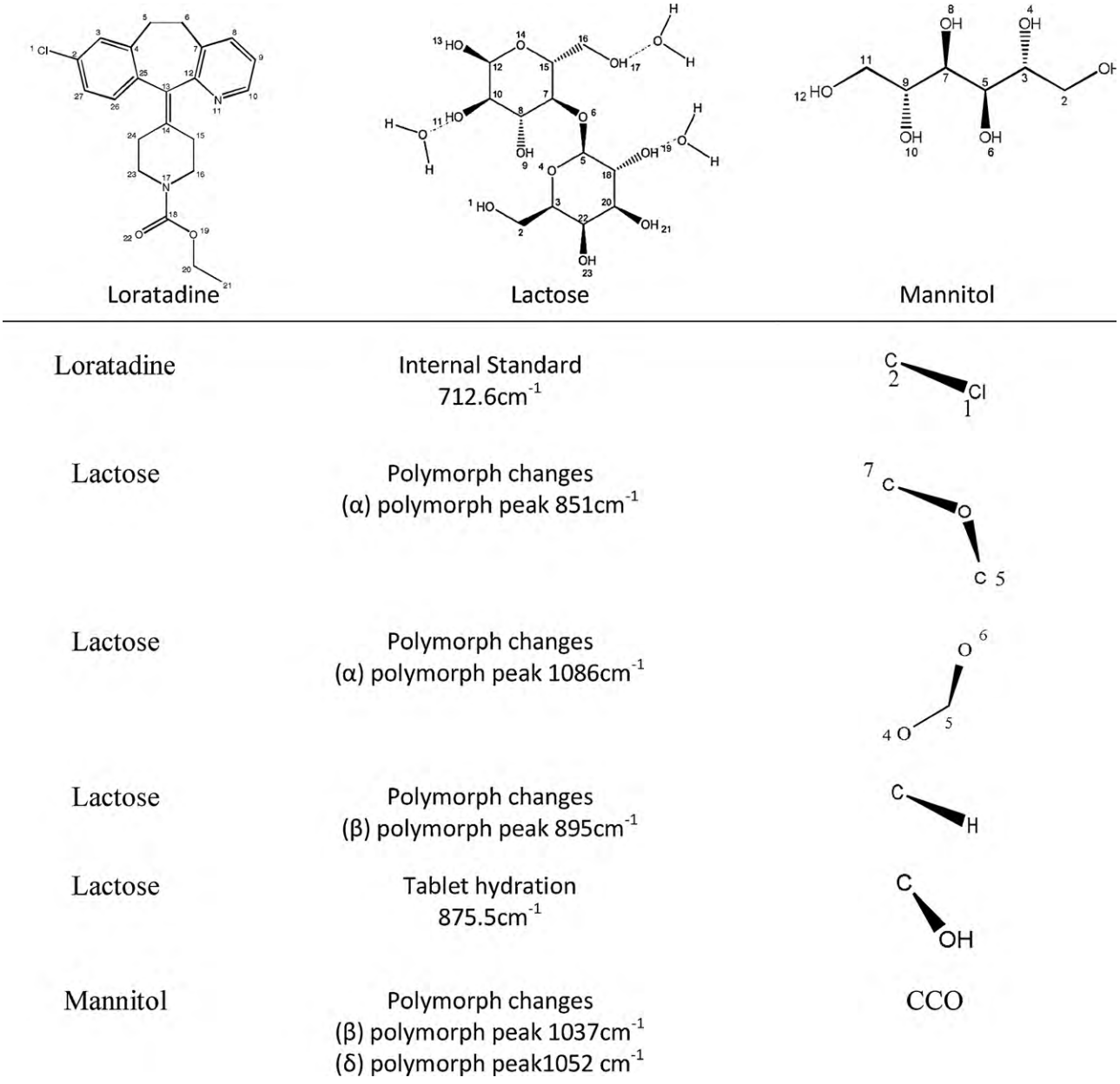

Fig. 1. CFGs of loratadine, lactose and mannitol used to determine surface water adsorption and polymorphic changes upon tablet surface hydration. The intensity of the C–Cl vibration of loratadine at 712.6 cm^{-1} is insensitive to water adsorption and was used as an internal standard during these studies; the intensity of the lactose, α glycosidic linkage vibration at 851 cm^{-1} and O–C–O vibration at 1086 cm^{-1} were used to study α lactose polymorphic changes; the C–H stretching at 895 cm^{-1} is a lactose β polymorph peak; the relative intensity of the C–OH stretching at 875.5 cm^{-1} decreases when water forms hydrogen bonds with this functional group; the 1037 cm^{-1} and 1052 cm^{-1} were used to study mannitol β and δ polymorphic forms, respectively.

The Raman spectrometer microscope was equipped with a CCD camera (128 × 256 pixels) which was used to visually image the tablet surface during the experiments. This camera was used to verify the focus position at the tablet surface and to record visual images of the surface for morphological changes before and after hydration.

Two methods were used to monitor adsorbed water on tablet surfaces: (i) an indirect measurement using Raman Spectroscopy and (ii) a direct measurement using a high sensitivity microbalance. The Raman method used the intensity of the C–OH stretching vibration at 875.5 cm^{-1}, which is expected to decrease in intensity when the functional group is hydrated. To verify this phenomenon, microdrops of water were placed on a freshly opened tablet. Raman spectra were collected before and after placing the drop on the tablet.

2.2.1. Indirect moisture measurement using Raman spectroscopy

Tablet surfaces were subjected to hydration under two test conditions: (i) 20 °C, 50% RH and (ii) 50 °C, 100% RH. This indirect method of monitoring surface water only evaluates the fraction of the tablet surface containing the Raman intensity sensitive C–OH groups on the tablet surface and does not evaluate the total water content on the tablet surface. Thus, if multiple layers of water exist on the tablet surface Raman spectroscopy cannot monitor the phenomena.

2.2.2. Direct moisture measurement using microbalance

Total water on tablet surfaces was measured using a high sensitivity Mettler MT5 microbalance—Toledo Inc. The tablet weight loss after incubating tablets for 180 min in a 50 °C, 100% relative humidity chamber is a direct measure of tablet sur-

face water adsorption prior to the room temperature desorption phase.

2.3. Raman spectra data acquisition and data processing

Raman spectra were obtained using a microscope and Kaiser's HoloMap™ software. Data processing (peak position, intensity, baseline correction, etc.) were performed using Grams AI version 8.0 software (Thermo, Inc.). The Raman microscope was equipped with both 5× and 20× objective. The target tablet surface area irradiated by the laser using a 5× objective was ~80–100 μm in diameter (manufacturer's specifications). Similar results were obtained with both 5× and 20× microscope objectives. The optical bench was an RXN1-785 Analyzer (Kaiser Optical Systems, Inc., Ann Arbor, MI) equipped with an excitation laser operating at 785 nm. The excitation cable was a 64.5 μm diameter cable and the photon collection cable receiving singles from the specimen was 100 μm in diameter. A holographic filter removed the background Rayleigh scattering during data acquisition. Data acquisition used an accumulation time of 20 s, 3 accumulations and a laser power setting of 400 mW. The optical throughput was ~30% from the spectrometer main bench to the microscope and specimen; the instrument's optical throughput was linear from 0 to 400 mW (measured with an Orion Laser power meter purchased from Kaiser Optical Inc.). This indicates that ~120 mW of laser power was impinging the tablet target area when the main bench was set to 400 mW of laser power.

Raman data obtained with Kaiser's HoloMap™ software were exported into Grams AI 8.0 data processing software. The peak position and intensity of the lactose $\bar{\nu}$ 851.0 cm^{-1} and $\bar{\nu}$ 875.5 cm^{-1} were obtained after baseline correction from $\bar{\nu}$ 910 cm^{-1} to $\bar{\nu}$ 840 cm^{-1}. Peak positions varied by less than 0.3 cm^{-1}.

Grams AI peak pick routine was used to identify the maximum peak height at the maximum peak position. The intensity of lactose and loratadine peaks in the Raman spectrum varies depending on the accumulation times, number of accumulations, laser power and instrument configuration and day to day variability. This unavoidable variability in data acquisition was normalized by using the intensity of loratadine's C–Cl peak intensity at $\bar{\nu}$ 712.6 cm^{-1} as an internal standard. Spectral intensities among the data slightly varied because (i) the tablet is slightly curved, (ii) the laser focal point on the tablet surface slightly varied, (iii) there is a distribution of surface exposed functional groups being measured on the tablet surface and (iv) the day to day variability in obtaining spectral data. These are many of the reasons for using an internal standard. When monitoring surface water hydration the ratio $I\bar{\nu}$ 875.5 $cm^{-1}/I\bar{\nu}$ 712.6 cm^{-1} was calculated; thus, the loratadine C–Cl peak intensity is being used to normalize the data for experimentally variable data. The laser was focused on the tablet surface by optimizing the signal received at the detector during continuous scanning. The loratadine C–Cl stretching vibration at $\bar{\nu}$ 712.6 cm^{-1} was used as an internal standard to eliminate variability in peak intensity among spectra. This C–Cl peak does not overlap with any lactose peaks. A drop of water placed on the Walvert tablet did not change the observed C–Cl stretching intensity. This indicates that water on the tablet surface does not change the intensity of the C–Cl stretching vibration. The loratadine C–Cl peak intensity was quantified after baseline correction from $\bar{\nu}$ 722 cm^{-1} to $\bar{\nu}$ 700 cm^{-1}.

The different lactose polymorphs used in this work include (i) the pure α polymorph, (ii) the β polymorph contaminated with 20% α polymorph, and (iii) the amorphous polymorph. Pure powder samples of each of these compounds were spread on a glass slide, and then slightly compressed by another slide to smooth the surface which minimized specular and diffuse reflectance and other sources of light scattering that decreases the Raman signals from being detected in the same optics that the laser light travels through to irradiate the sample. Pure loratadine and mannitol spectra were collected under the same conditions.

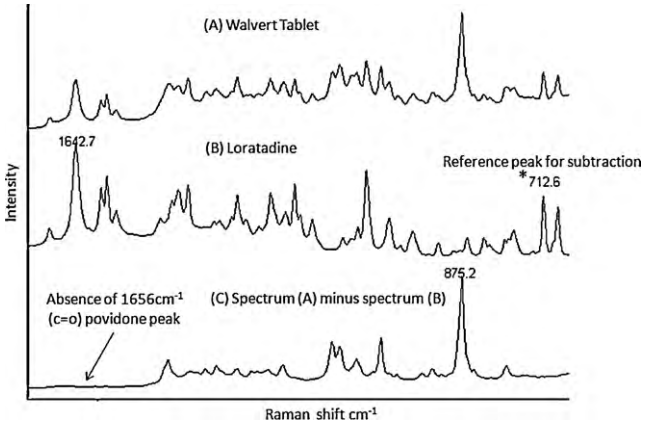

Fig. 2. (A) Walvert tablet spectrum, (B) powder loratadine spectrum, (C) spectral subtraction of spectrum (A) minus (B) using loratadine *712.6 cm^{-1} peak as reference peak. The figure indicates the absence of povidone carbonyl peak signifying no detectable povidone on the tablet surface.

2.4. Inter-tablet TSM variability

The distribution of the 875.5 cm^{-1} alcohol groups was evaluated. The statistical variable was the ratio of $I\bar{\nu}$ 875.5 $cm^{-1}/I\bar{\nu}$ 712.6 cm^{-1} and 48 tablets were used. The mean of three surface spectra on each tablet were used in the study. The Shapiro-Wilk test supports that the variable is normally distributed. The critical value of the Shapiro-Wilk test statistic is $W_{0.05}(48) = 0.947$ and the test statistics are

$$W = \frac{\left[\sum_{i=1}^{24} a_i(x_{(49-i)} - x_{(i)})\right]^2}{\sum_{i=1}^{48}(x_{(i)} - \bar{x})^2} = \frac{5.126612^2}{27.375931} = 0.960$$

where $x_{(i)}$ are ordered data and a_i are the Shapiro–Wilk coefficients. Since $W = 0.960$, which is >0.947, the probability that the ratio of alcohol groups is normally distributed based on the Raman intensity ratio of $I\bar{\nu}$ 875.5 $cm^{-1}/I\bar{\nu}$ 712.6 cm^{-1} is derived from a normal distribution is statistically valid. Inter-tablet variability of lactose alcohol TSMs is normally distributed.

3. Results

The amount of the inactive ingredients in commercial products is proprietary information. Low concentration excipients not observable in the Raman spectra of Walvert tablets include (i) artificial cherry flavor, (ii) aspartame, (iii) colloidal silicon dioxide, (iv) sodium lauryl sulfate and (v) sodium stearate. More detailed studies were performed to identify the approximate amount of lactose monohydrate, mannitol, povidone and microcrystalline cellulose.

Fig. 2 demonstrates that povidone is not observable in the Raman spectra of Walvert tablets. The spectral subtraction shows a baseline Raman peak intensity at 1656 cm^{-1}, where povidone normally elicits a strong peak intensity (Fig. 2(C)). This indicates that povidone is a low concentration excipient in Walvert tablets.

In the Raman tablets spectra, the 1095 cm^{-1} and 1292 cm^{-1} peaks appear as a considerably weak peak and shoulder, respectively. Based on this data, the concentration of microcrystalline cellulose is minimal in the analyzed tablets. In addition, these microcrystalline cellulose peaks, do not interfere with the peaks of interest used in our analysis (875.5 cm^{-1}, 851.0 cm^{-1}, and 712.6 cm^{-1}).

Upon studying the spectra of the Walvert tablets, mannitol and lactose, it appears that the major excipients in the tablets

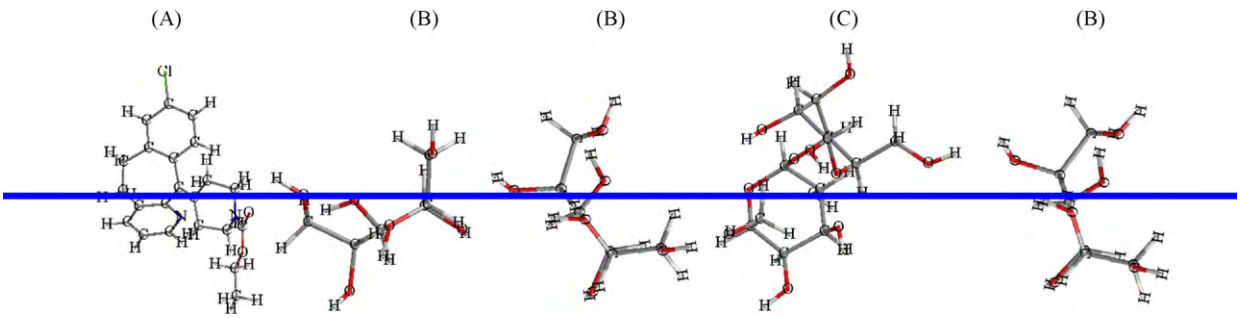

Fig. 3. Scheme showing possible heterogeneous orientations of loratadine (A) at a hypothetical tablet surface, mannitol (B) in different orientations and lactose (C).

are mannitol and lactose (Fig. 1). Based on the mannitol lactose monohydrate calibration curve, the ratio of lactose monohydrate to mannitol in the Walvert tablets was approximately between 1:3 and 1:4.

The chemical functional groups of the API and excipients in Walvert tablets used in this study are shown in Fig. 1. The chemical structures of mannitol, lactose and loratadine and the Raman vibrations used to monitor (i) tablet surface hydration and (ii) the Raman vibrations used to monitor the infrequent inter conversion of lactose and mannitol polymorphs when tablet surfaces are hydrated are illustrated in Fig. 1. The existence of the API and excipient molecules in different conformations at the tablet surface, affects the availability of the CFGS for hydration (Fig. 3).

The changes in Raman peak intensities of C–OH CFGs and loratadine C–Cl stretching vibrations when water is applied to the tablet surface are illustrated in Fig. 4. The key findings are (i) the tablet excipient lactose alcohol C–OH peak at 875.5 cm^{-1} decreased to virtually 80% or more of the non hydrated stated, and (ii) the tablet loratadine C–Cl peat at 712.6 cm^{-1} peak did not change intensity when the tablet is hydrated with a drop of water on the surface. Thus, the loratadine C–Cl stretching frequency was useful as an internal standard during surface measurements of tablets.

The residual peak intensity at 875.5 cm^{-1} in the hydrated tablet spectrum (Fig. 4(B)) is indicative of residual alcohols not accessible to surface hydration. Raman laser penetrates the tablet to a depth

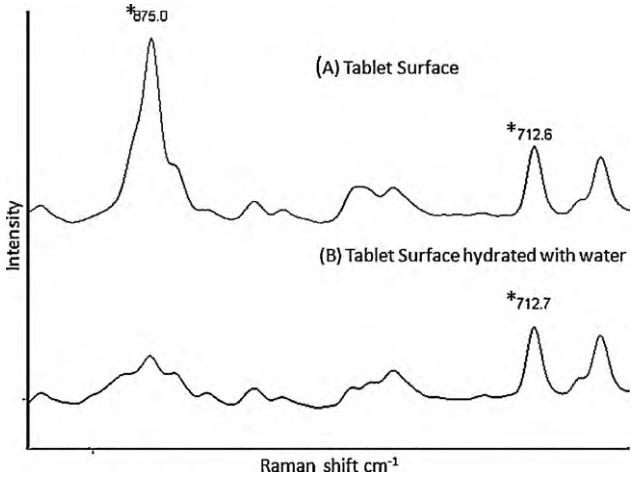

Fig. 4. Experimental determination of Raman active vibrations that decrease in intensity when tablets are hydrated in vapor phase water at elevated temperatures. Raman spectrum (A) was obtained from a Walvert tablet containing the API loratadine. Raman spectrum (B) was obtained after microdrops of water were placed on the tablet surface. The intensity of the loratadine C–Cl stretching frequency at *712.6 cm^{-1} did not change, whereas the alcohol C–OH stretching intensity significantly decreased in the hydrated tablet *875.5 cm^{-1} in the spectra (B) is indicative of residual alcohols not accessible to surface hydration.

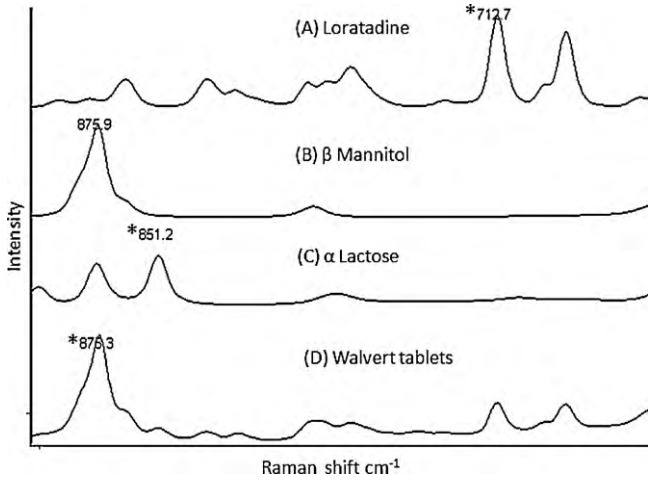

Fig. 5. (A) Raman Spectrum of pure loratadine showing strong *712.6 cm^{-1} vibration assigned to the C–Cl. (B) The Raman spectrum of mannitol showing peak at *875.5 cm^{-1} common with α lactose and detected on the Walvert tablets. (C) Raman spectra of α lactose demonstrating α specific peak at *851.0 cm^{-1}. (D) The Raman surface spectrum of a Walvert tablet containing loratadine as the API and excipients mannitol and lactose. The relative intensity ratio of the lactose *875.5 cm^{-1} to the loratadine *712.6 cm^{-1} vibration are apparent in the figure.

slightly more than can be hydrated in these studies. Nevertheless, the decrease in intensity of the C–OH stretching frequency was used to qualitatively monitor water on the tablet surface.

The Raman spectra of (i) powdered loratadine, (ii) α lactose and (iii) β mannitol and (iv) commercially available Walvert tablet are shown in Fig. 5. The loratadine C–Cl peak at 712.6 cm^{-1} and the alcohol peak at 875.5 cm^{-1} reside in distinct regions of the spectrum. This indicates that there are no overlapping Raman peaks and both the Raman active C–OH alcohol peak and loratadine C–Cl peak can be monitored without the problem of overlapping peaks from other functional groups.

The inter-tablet variability in the distribution of surface alcohol groups was evaluated. Fig. 6 is the experimentally measured frequency distribution of the inter-tablet variability of C–OH alcohol groups on the Walvert tablet surface. The inter-tablet distribution of the alcohol groups showed normal distribution ($p < 0.05$) with a mean of 3.22 and sample standard deviation of 0.227 (Table 1).

Table 1
Comparison of the PDF (Fig. 6) to the first experimental time points of the tablets being hydrated or incubated directly at room temperature.

	$I\bar{\nu}^{-1}$ 875.5 cm^{-1} / $I\bar{\nu}$ 712 cm^{-1}	
	Mean	Standard deviation
Frequency distribution function (Fig. 6)	3.22	0.277
Tablets (Fig. 7(B))	3.11	0.524

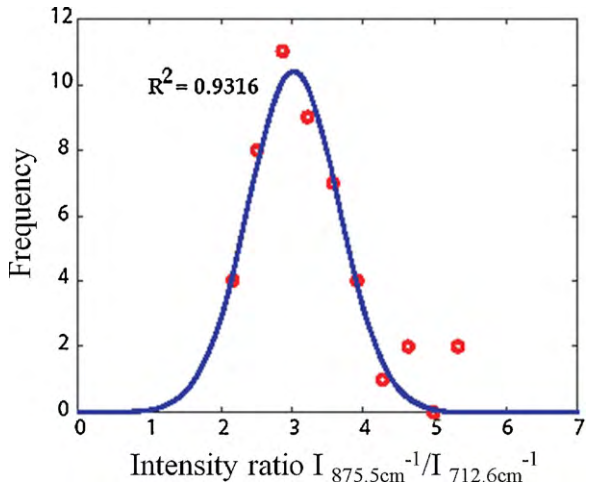

Fig. 6. Shows the normal distribution of the intensity ratio of the 875.5 cm^{-1} in reference to 712.6 cm^{-1} upon testing 48 tablets.

The frequency distribution of alcohol functional groups and loratadine C–Cl functional groups, in non hydrated Walvert tablets enabled us to determine the Walvert inter-tablet variability of C–OH groups distribution, i.e. the TSMs and loratadine C–Cl CFG. Although we used a ratio of the intensity of these functional group vibrations, the normal distribution shown in Fig. 6 and Table 1 reflects the distribution of TSM CFGs.

3.1. Indirect moisture measurement using Raman spectroscopy

Four control tablets and seven test tablets were used to generate this data. The room temperature and humidity conditions did not result in surface hydration of the control tablets. This is evident by the straight lines in the $I\bar{\nu}\,875.5\,cm^{-1}/I\bar{\nu}\,712.6\,cm^{-1}$ ratio shown in Fig. 7(A). This data indicates that during the manufacturing of Walvert tablets at room temperature and humidly conditions, there is little to no water adsorption on the tablet surfaces prior to packaging in blister packs. However, a significant hydration event of the tablet surface occurred at 50 °C and 100% relative humidity. The ratio of $I\bar{\nu}\,875.5\,cm^{-1}/I\bar{\nu}\,712.6\,cm^{-1}$ decreased from 3.11 ± 0.524, before incubation in the humidifier to 0.883 ± 0.346 after 180 min of incubation (Fig. 7(B)). Upon removing the tablets from the humidity chamber, the $I\bar{\nu}\,875.5\,cm^{-1}/I\bar{\nu}\,712.6\,cm^{-1}$ ratio increased to 1.71 ± 0.494 over 90 min. The $I\bar{\nu}\,875.5\,cm^{-1}/I\bar{\nu}\,712.6\,cm^{-1}$ ratio did not return to the pre hydration ratio.

3.2. Direct moisture measurement using microbalance

Water molecules are ~2.9 Å in diameter and the surface area of water is ~6.6 $Å^2$. The ~14 mg of water adsorbed to the tablet surface area corresponds to ~0.77 mMole of water (14 mg (H_2O)/18 mg $mMole^{-1}$(H_2O) is 0.77 mMole (H_2O)). Given the approximation that 0.77 mMole (H_2O) was adsorbed to the tablet surface, then 0.77 mMole multiplied (by Avogadro's number) 6.023×10^{21} molecules per mMole are 4.63×10^{21} molecules of H_2O on the tablet surface. The tablet diameter is ~6.4 mm and a thickness of 2.53 mm, thus the tablet surface area is ~83 mm^2. Based on the amount of water adsorbed to the tablet surface, the ~6.6 $Å^2$ surface area of water can form approximately $\sim 1.25 \times 10^{15}$ water layers on the tablet surface. This is an overestimate of the number of layers of water on the tablet surface because water can penetrate the tablet itself.

The direct measurement of water desorption (Fig. 8(A), $t > 180$ min) showed an exponential decay (Fig. 8(B)). This indicates that an exponential decay of water desorption from the tablet surface is proportional to the surface concentration of adsorbed water, which had accumulated during the incubation period. This exponential decay of water was not observed when using Raman measurements and hydrated lactose alcohols as a Raman signal. This is obvious in Fig. 7(B) where irreversibly bound water shown by the plateau at ratio of $I\bar{\nu}\,875.5\,cm^{-1}/I\bar{\nu}\,712.6\,cm^{-1}$ lower than the initial value. The plateau is indicative of tightly bound water to the tablet surface (based on Raman spectroscopy). Whereas Fig. 8 shows an exponential decay or desorption of water on the tablet surface based on a non-specific gravimetric analysis of tablet surface water. Thus, gravimetric method data is monitoring the loss of the multiple layers of non-specific water binding on the tablet surface that accumulated during the incubation period. The Raman method is clearly demonstrating that not all of the water layers desorbs according to a simple exponential function. Based on Fig. 8(B), the log mean weight change = $-0.004t + 1.864$, $R^2 = 0.991$, and the desorption half-life, $t_{1/2}$, was ≈70 min. This observation represents the bulk water adsorbed followed by desorption and does not represent the water adsorbed to the tablet surface alcohol functional groups that tightly bind water as measured by Raman spectroscopy.

Lactose is known to undergo polymorph changes upon hydration [27]. Tablet surface hydration can stimulate lactose to convert from amorphous to the α polymorph, which was observed in this

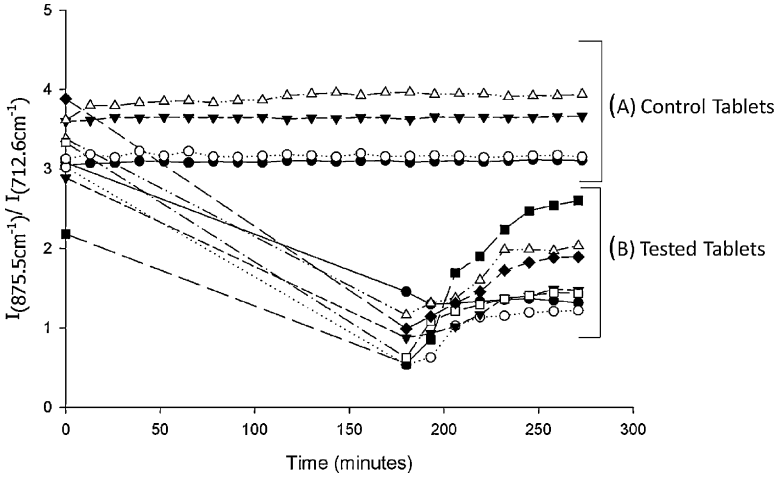

Fig. 7. Raman spectroscopy analysis of control tablets incubated at room temperature (A), and test tablets stored at 50 °C with 100% relative humidity (B).

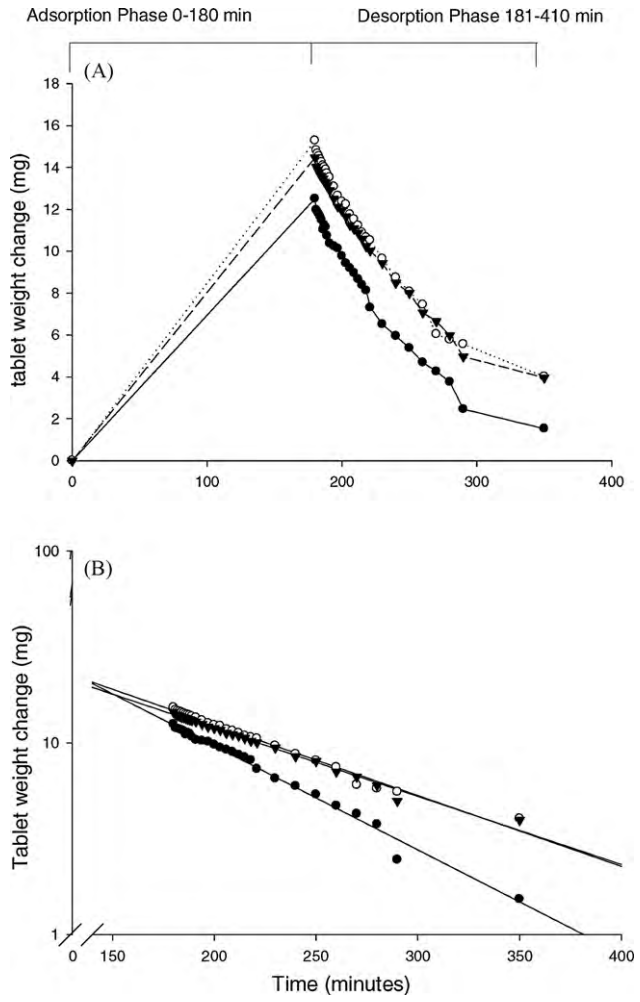

Fig. 8. (A) Change in tablet weight with time during the desorption phase (181 min to 410 min) from the tablet surface at 20 °C and 50% RH. (B) A log plot of (A) was used to estimate the number of possible water adsorbed layers and also the $t_{1/2}$ of water desorption.

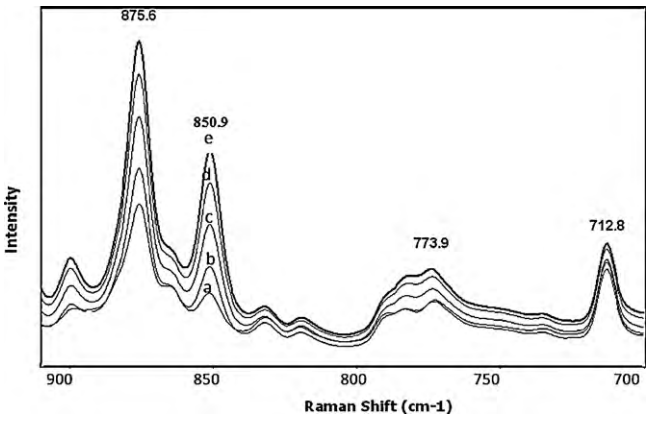

Fig. 9. Formation of the α lactose polymorph identified by the intensity increase of the $I\bar{\nu}$ 850.9 cm^{-1} / $I\bar{\nu}$ 712.8 cm^{-1} ratio. The Raman peak at 850.9 cm^{-1} is characteristic of the α lactose polymorph and an increase in this peak intensity is indicative of an increase in the amount of the α lactose polymorph in the tablet. Spectra were generated after the following incubation conditions at 50 °C and 100% relative humidity: (a) 0 min; (b) 15 min; (c) 30 min and (d) 45 min (e) 60 min. Visual comparison of spectra (a) and (e) indicates that the α lactose polymorph peak at 850.9 cm^{-1} increases ~10-fold compared to the increase in the Raman peak intensity at 875.6 cm^{-1} corresponding to the lactose monohydrate crystalline material which only increases in intensity approximately 3 folds.

study (Fig. 9). Polymorphic changes were observed in two out of the ten tablets tested, which indicates that this is a low probably event, but nevertheless it is a real physical change at the tablet surface when hydrated. Fig. 9 shows the spectral data demonstrating the transition from the amorphous to the α lactose polymorph. The amorphous lactose converting to the α lactose was monitored using both the C–O–C vibration (851.0 cm^{-1}) and the O–C–O vibration (1086 cm^{-1}) [not shown in the figure] specific to α lactose. An increase in the 851.0 cm^{-1} peak intensity was observed over time. The lactose peak at 895.6 cm^{-1} is characteristic to β lactose polymorph [17,28,29]. No β lactose was observed during this study.

The tablets contain β mannitol polymorph, the most thermodynamic stable form. This is based on the presence of the 1037 cm^{-1} peak, CCO stretching vibration [35,36]. The absence of 1052 cm^{-1} peak, significant to δ mannitol, in the tablet spectra, before and after incubation, signifies that mannitol does not undergo polymorphic changes.

4. Discussion

The TSM concept demonstrated in this study can be applied to many commercially manufactured tablets. TSM is a concept that allows probing of tablet surfaces for pharmaceutical or chemical phenomena that is a physical observable elicited from the CFGs from either the excipients or API. It is likely that all tablets have chemical functional groups on their surfaces, which may be useful as chemical probes to determine pharmaceutical properties of tablet surfaces.

Raman analysis of tablet specimens requires virtually no sample preparation. The advantages of Raman spectroscopy are that the method is noninvasive and nondestructive[30–33]. The use of Raman spectroscopy to study water–excipient interactions is more useful than IR spectroscopy because water elicits strong IR intensities over a broad range of wavelengths. This may mask many chemical functional groups or saturate the detector signal. Water has little to no Raman active vibrations.

After incubation in a humidity chamber at 50 °C our experiments demonstrated that Walvert tablets exhibit at least two states of surface adsorbed water (i.e., loosely and tightly bound); the plateau region demonstrated that tablet surface water is tightly bound (Fig. 7(B)). It is clear that the tablet surface water did not completely evaporate as evidenced by the plateau region not returning to pre incubation intensity ratio value. The release or evaporation of adsorbed water occurs at an exponential rate for the loosely bound water (Fig. 8); whereas, the tightly bound water (Fig. 7(B)) is apparently irreversible at room temperature. This is evident in Fig. 7(B) where there was water loss from the tablet surfaces for about 90 min after the 180 min incubation period.

If only a direct measurement of total tablet water was experimentally performed, using gravimetric analysis, the observation that a sub population of alcohol hydrogen binding sites on the tablet surface would not have been observed; Raman spectroscopy was needed to observe the tightly bound water state.

This suggests that alcohols on the tablet surface may exist in different chemical environments for example the alcohol may be (i) already H-bonded to a neighboring molecule within the tablet and/or (ii) oriented in a sterically hindered position in the solid state tablet. These chemical parameters of alcohol CFGs control the signal observed when monitoring hydrated and non-hydrated tablet surface alcohols. Another consideration is that the laser used to obtain Raman spectra likely penetrates beyond where the hydration occurs; non-hydrated alcohols inside the tablet can thus be

recorded when attempting to monitor the tablet surface hydration.

When a few water molecules adsorb to the tablet surface at specific sites (e.g., lactose alcohol sites), these water molecules may facilitate the binding of additional water on the tablet surface. From the gravimetric analysis we observed enough water adsorption during incubation to form thousand of layers. Apparently a small thickness of a water layer resides at the tablet surface when tablets are placed in a 50 °C humidifier at 100% relative humidity for 3 h; this bulk water exponentially desorbs from the tablet surface (Fig. 8(B)).

Experimentally we used a 20× objective, which caused the laser impinging the tablet surface to have approximately an 80–100 μm diameter target area. Based on the molecular size of the excipients and API, a laser beam of 80–100 μm diameter focused on the tablet surface irradiates thousands of molecules. Thus, the Raman measurements are recording an average distribution of the C–OH TSMs relative to the C–Cl internal standard.

We measured a Gaussian distribution of the ratio of I 875.5 cm^{-1}/I 712.6 cm^{-1} using Raman spectroscopy (Fig. 6). Since the statistical variable is a ratio, both the distribution of the TSMs associated with the 875.5 cm^{-1} (alcohols) and the internal standard associated with the 712.6 cm^{-1} peak (loratadine) exhibit variance. The variance in the distribution of both molecules on the tablet surface can change the observed ratio.

Mannitol is a monosaccharide with alcohol CFGs that elicit Raman active vibrations in the same spectral region as lactose, i.e. 875.6 cm^{-1}. Nevertheless, mannitol is non hygroscopic [34], but dissolves in the presence of water and this causes changes in the Raman spectrum of the solution state mannitol. In solid state phase, mannitol does not easily hydrate. The Raman spectrum of the mannitol alcohols is less likely to change upon hydration. Thus the 875.6 cm^{-1} peak changes are most likely attributed to lactose hydration.

5. Conclusions

TSMs may be useful in evaluating counter fit tablets and for process analytical techniques (PAT) during tablet manufacturing. However, implementing this technique requires an understanding of the tablet surface chemistry relative to TSMs that probe specific chemical properties or events at the tablet surface. TSMs may likely be specific to each tablet composition and manufacturing process. The Raman method described in this work using alcohol TSMs can be incorporated in the design of a PAT to monitor the tablet drying end point during manufacturing.

Acknowledgments

The authors wish to thank Massachusetts College of Pharmacy and Health Sciences for providing funding and access to the lab equipment and Xiang Qian Chang PhD, for his tremendous help with the statistics through our study.

References

[1] P. Matousek, A.W. Parker, Appl. Spectrosc. 60 (2006) 1353–1357.
[2] P. Corti, G. Ceramelli, E. Dreassi, S. Mattii, Analyst 124 (1999) 755–758.
[3] X.S. Zhao, J.I. Siepmanii, W. Xu, Y. Kiang, A.R. Sheth, S. Karaborni, J. Phys. Chem. B 113 (2009) 5929–5937.
[4] Y. Xie, W. Tao, H. Morrison, R. Chiu, J. Jona, J. Fang, N. Cauchon, Int. J. Pharm. 362 (2008) 29–36.
[5] S. Mazurek, R. Szostak, J. Pharm. Biomed. Anal. 49 (2009) 168–172.
[6] C.J. Strachan, T. Rades, K.C. Gordon, J. Rantanen, J. Pharm. Pharmacol. 59 (2007) 179–192.
[7] M. Sheikhzadeh, S. Rohani, A. Jutan, T. Manifar, K. Murthy, S. Horne, Pharm. Res. 23 (2006) 1043–1050.
[8] A. Caillet, F. Puel, G. Fevotte, Int. J. Pharm. 307 (2006) 201–208.
[9] A. Caillet, A. Rivoire, J. Galvan, F. Puel, G. Fevotte, Cryst. Growth Des. 7 (2007) 2080–2087.
[10] S. Šašić, M. Whitlock, Appl. Spectrosc. 62 (2008) 916–921.
[11] N.F. Abu Bakar, A. Mujumdar, S. Urabe, K. Takano, K. Nishii, M. Horio, Powder Technol. 176 (2007) 137–147.
[12] S. Šašić, Pharm. Res. 24 (2007) 58–65.
[13] L. Zhang, M.J. Henson, S.S. Sekulic, Anal. Chim. Acta 545 (2005) 262–278.
[14] H. Seager, J. Pharm. Pharmacol. 50 (1998) 375–382.
[15] M. Cirri, C. Rangoni, F. Maestrelli, G. Corti, P. Mura, Drug Dev. Ind. Pharm. 31 (2005) 697–707.
[16] Y. Fu, S. Yang, S.H. Jeong, S. Kimura, K. Park, Crit. Rev. Ther. Drug Carrier Syst. 21 (2004) 433–475.
[17] J.H. Kirk, S.E. Dann, C.G. Blatchford, Int. J. Pharm. 334 (2007) 103–114.
[18] M.E. Auer, U.J. Griesser, J. Sawatzki, J. Mol. Struct. 661–662 (2003) 307–317.
[19] C. Ahlneck, G. Zografi, Int. J. Pharm. 62 (1990) 87–95.
[20] G. Zografi, Drug Dev. Ind. Pharm. 14 (1988) 1905–1926.
[21] A.K. Salameh, L.S. Taylor, J. Pharm. Sci. 95 (2006) 446–461.
[22] B.M. Murphy, S.W. Prescott, I. Larson, J. Pharm. Biomed. Anal. 38 (2005) 186–190.
[23] D.L.A. De Faria, H.A.C. Gil, A.A.A. De Queiróz, J. Mol. Struct. 478 (1999) 93–98.
[24] G.P. Andrews, O.A. Abudiak, D.S. Jones, J. Pharm. Sci. 99 (2010) 1322–1335.
[25] P.M. Fechner, S. Wartewig, M. Füting, A. Heilmann, R.H.H. Neubert, P. Kleinebudde, AAPS Pharm. Sci. 5 (2003).
[26] R. Rowe, P. Sheskey, M. Quinn, Handbook of Pharmaceutical Excipients, sixth ed., Pharmaceutical Press, Chicago, 2009.
[27] A. Flemming, K.M. Picker-Freyer, Eur. J. Pharm. Biopharm. 68 (2008) 802–810.
[28] L. Nørgaard, M.T. Hahn, L.B. Knudsen, I.A. Farhat, S.B. Engelsen, Int. Dairy J. 15 (2005) 1261–1270.
[29] P.A.M. Williams, S.B. Etcheverry, E.J. Baran, Carbohydr. Res. 329 (2000) 41–47.
[30] C. Eliasson, P. Matousek, Anal. Chem. 79 (2007) 1696–1701.
[31] C. Eliasson, P. Matousek, Am. Lab. 39 (2007), 42–44 + 10.
[32] M.G. Orkoula, C.G. Kontoyannis, J. Pharm. Biomed. Anal. 47 (2008) 631–635.
[33] S. Virtanen, O. Antikainen, J. Yliruusi, Int. J. Pharm. 360 (2008) 40–46.
[34] T. Yoshinari, R.T. Forbes, P. York, Y. Kawashima, Int. J. Pharm. 247 (2002) 69–77.
[35] S. Campbell, A. Williams, I. Grimsey, J. Pharm. Biomed. Anal. 28 (2002) 1135–1147.
[36] Y. Xie, W. Cao, S. Krishnan, H. Lin, N. Cauchon, Pharm. Res. 25 (2008) 2292–2301.