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Research Article

Theme: Team Science and Education for Pharmaceuticals: the NIPTE Model
Guest Editors: Ajaz S. Hussain, Kenneth Morris, and Vadim J. Gurvich

New Insights on Solid-State Changes in the Levothyroxine Sodium Pentahydrate during Dehydration and its Relationship to Chemical Instability

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Abstract. Levothyroxine sodium pentahydrate (LEVO) tablets have been on the US market since the mid-twentieth century and remain the most highly prescribed product. Unfortunately, levothyroxine sodium tablets have also been one of the most highly recalled products due to potency and dissolution failures on stability. In 2008, the assay limits were tightened, yet the recalls did not decline, which highlights the serious quality concerns remaining to be elucidated. The aim of the present investigation was to test the hypothesis that the solid-state physical instability of LEVO precedes the chemical instability leading to product failure. The failure mode was hypothesized to be the dehydration of the crystal hydrate, when exposed to certain humidity and temperature conditions, followed by the oxidation of the API through vacated channels. It was previously reported by the authors that LEVO degradation occurred in the presence of oxygen at a low relative humidity (RH). Furthermore, powder X-ray diffractometry shows changes in the crystal lattice of LEVO initially and through the dehydration stages. Storage of LEVO at RT and 40 °C at 4–6% RH for 12 days shows a decrease in d-spacing of the (00 l) planes. Based on a structure solution from the powder data of the dehydrated material, the basic packing motif persists to varying degrees even when fully dehydrated along with disordering. Therefore, the crystal structure changes of LEVO depend on RH and temperature and are now explicable at the structural level for the first time. This exemplifies the dire need for "new prior knowledge" in generic product development.

KEY WORDS: crystal structure; hydrate; powder X-ray diffraction; new prior knowledge; levothyroxine.

INTRODUCTION

Levothyroxine sodium pentahydrate (LEVO) is a man-made thyroid hormone chemically identical to thyroxine, the hormone naturally made by the thyroid gland to restore thyroid hormone balance (1). The synthetic form of thyroxine, USP was first available in the 1950s (2). LEVO remained an unapproved marketed product until 1997 (3), when instances of sub or super potency of the drug products were reported. In an effort to overcome problems associated with the LEVO tablets, USFDA declared LEVO a "new drug" in 2001 (4). Despite thorough review by the agency, the potency issues with LEVO did not improve. Next, in 2008, the United States Food and Drug Administration (USFDA) tightened the assay limits for LEVO tablets from 90.0–110.0% to 95.0–105.0% (5), which mainly resulted in shorter shelf life. The cause of instability in LEVO tablets remained unclear for almost half a century.

Hamad *et al.* (6) concluded that LEVO degrades in presence of oxygen especially when the crystalline material is

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exposed to low humidity conditions (dehydrated form). Patel *et al.* (7) suggested the possible reasons for degradation of LEVO were (1) use of different excipients, (2) pH of the formulation, and (3) compression force used during tablet compaction. Khan *et al.* (8) concluded that a careful selection of excipients could be helpful to prevent potency loss over the shelf life of the tablets of LEVO. Byrn *et al.* reported that some APIs exhibit degradation only following desolvation (9).

Despite numerous reports (6,10–29), the primary issue of physical-chemical instability link was never elucidated. The physical-chemical stability and related dosage form performance of many products, especially narrow therapeutic index drugs, depends upon the integrity of the solid state (15,30–44). Insufficient characterization and lack of understanding of the solid state may contribute to failures during drug product development and unidentified root causes for failures in the clinic (45–47).

Due in part to a very low dose of the drug, there is little reported interpretation of the earlier studies that reflects an understanding of the solid-state behavior of LEVO in the dosage form dominated by excipients. In particular, the influence of removal of water from the crystal lattice of LEVO is unclear. The United States Pharmacopeia describes levothyroxine sodium as a pentahydrate (48). This pentahydrate form might lead to a series of very closely related crystal structures upon removal of water molecules, which was hypothesized to allow access to molecular oxygen into the crystal lattice of LEVO.

A report from Hamad *et al.* demonstrated the oxidative degradation of LEVO at low humidity and the investigation is continued in the present study (6). The aim of the present investigation is to elucidate the solid-state properties of LEVO at the crystallographic level as it changes hydration state. From powder X-ray diffractometry (PXRD) and thermal analysis data, it can be concluded that storage at low humidity and high temperature leads to the formation of a dehydrated crystal structure.

The current research is also intended to serve as an example of the application of the generation and use of "new prior knowledge" (49–51) in the development of generic drug products of levothyroxine sodium as well as products with physiochemically similar APIs. Ultimately, maximizing the use and acceptance of new prior knowledge can be a powerful tool to support and accelerate the CMC development for pharmaceutical products such as levothyroxine sodium.

EXPERIMENTAL

Materials

LEVO was obtained by courtesy from Mylan Pharmaceuticals and Lupin Pharmaceuticals and stored in sealed polyethylene bags in a −15 °C freezer. Methanol (HPLC grade) was obtained from Pharmaco-AAPER. (Shelbyville, KY), acetonitrile (HPLC grade) from Pharmaco-AAPER. (Shelbyville, KY), trifluoroacetic acid (HPLC grade) was from Sigma-Aldrich (St. Louis, MO) and sodium hydroxide (N.F. FCC grade) from J.T. Baker Chemical Co. (Phillipsburg, NJ). Drierite® (anhydrous calcium sulfate) was from W.A. Hammond drierite company LTD (Xenia, OH). Water was prepared using a Milli-Q Direct 8 water purification system (EMD Millipore, Billerica, MA).

Sample Treatment at Different Temperature and Relative Humidities for Solid-State Analysis

LEVO was placed in a glass petri dish (approximately 100 mg) and in 2 mm X-ray aluminum cell holder (approximately 600 mg). RH conditions were chosen with the goal of dehydration at 0% RH and hydration at 100% RH of the crystal hydrate. Four to six percent RH conditions were produced using drierite and water was used for 100% RH conditions. The drierite and water were kept in Thermo Scientific Nalgene autoclavable plastic desiccators with a 230-mm plate on which the LEVO samples were placed. The desiccator was then tightly sealed and allowed to equilibrate at RT or 40 °C. The RT conditions were maintained by placing desiccators in a closed cabinet to avoid any photodegradation in an air-conditioned laboratory. Forty degree Celsius was maintained by placing the desiccators in a hot air oven (DK-63 Baxter Scientific Products, Deerfield, Il). The temperature and RH in the desiccators were monitored using AcuRite® digital temperature and humidity monitors (model # 01083, Chaney Instruments Co., Lake Geneva, WI). At 0-, 3-, 6-, and 12-day time points, the sample was removed and PXRD patterns were collected. At the end of the 12-day study, thermal analysis, moisture sorption, and Karl Fisher titrimetry were performed.

Thermogravimetric Analysis

A thermogravimetric analyzer (Q500, TA Instruments, White Castle, DE) was used to determine % moisture loss from LEVO initially and exposed to 100% RH ($LEVO_H$) and to 4–6% RH ($LEVO_{DH}$). Samples weighing approximately 3–5 mg were placed in a hermetic aluminum pan with a pinhole. Thermogravimetric analysis (TGA) was carried out at a ramp rate of 10 °C/min from 25 to 250 °C with a dry nitrogen purge at 50 ml/min.

Differential Scanning Calorimetry

A differential scanning calorimeter (Q2000, TA Instruments, White Castle, DE) was used to measure heat flow associated with physicochemical transitions of LEVO as a function of temperature. Sample weights (approximately 2–4 mg) were placed in a hermetic aluminum pan with a pinhole. An empty hermetic aluminum pan was used as a reference. Differential scanning calorimetry (DSC) was carried out at a ramp rate of 10 °C/min from 25 to 250 °C with a dry nitrogen purge at 50 ml/min. The DSC was calibrated for temperature and heat capacity using indium and sapphire respectively.

Moisture Sorption

Water uptake by LEVO at 25 °C was studied using a moisture sorption (MS) analyzer (Q5000, TA Instruments, White Castle, DE). The relative humidity of the nitrogen over the sample was controlled computer, which sets the appropriate flow to the wet side (100% relative vapor pressure of water) and dry side (dry nitrogen). Approximately 3–5 mg samples were placed directly into the quartz sample cup, which was loaded onto one side of the twin pan balance. TA

instrument measurement system was used to analyze and process the data. To study the sorption profile of LEVO, $LEVO_H$, and $LEVO_{DH}$ as a function of RH in the range 5–95%, the RH after equilibration was increased to 95% in steps of 10% and the water sorption was monitored. The RH was increased to the next step when the weight of the sample was constant. Nitrogen was purged at a total flow rate of 200 mL/min.

Karl Fischer Titrimetry

The water content of LEVO was determined in triplicate using a Coulometric Moisture Meter (787 KF Titrino, Riverview, FL). LEVO initial and exposed to 100% RH and to 4–6% RH were accurately weighed by difference and quickly transferred to the analyte chamber. Hydranal® (methanol) was used for the analysis.

Powder X-ray Diffractometry

Powder X-ray diffractometry (PXRD) patterns were obtained using a SmartLab® wide-angle X-ray diffractometer (Rigaku Corporation, Austin, TX). Cu K-α radiation was generated at 44 kV and 40 mA. LEVO (500–600 mg) was placed in 2 mm deep aluminum cell and leveled with a glass slide. The samples were scanned from 4 to 40° 2θ at a step size of 0.01°, and a scan speed of 1°/min with a spin rate of 15 rpm in the Bragg Brentano geometry. For variable temperature PXRD studies, sample (50–60 mg) was placed between Kapton film in a linkam hotstage and PXRD data was collected in vertical transmission mode. The linkam stage was leveled with a glass slide. The samples were scanned from 4 to 40° 2θ at a step size of 0.04° and a scan speed of 3°/min.

Quantitative Method for % Crystallinity Calculation Using Excel Full Pattern Fitting

A quantitative PXRD method was developed utilizing a full pattern fitting method in Microsoft Excel. The amorphous model was developed by using measured reference patterns for the LEVO and $LEVO_{DH}$. The scale factors for each of the normalized reference patterns were optimized using the Excel Solver (GRG nonlinear) function to minimize the difference between calculated and measured PXRD data. All measured and reference data used in the method were collected under the same conditions and were initially background subtracted to remove the instrumental contribution and subsequently normalized to give a constant integrated intensity. By adjusting the individual scale factors, the Excel Solver (GRG nonlinear) function minimized the difference between the calculated model data and unknown data. The returned scale factors were used as quantitative measures of the % crystallinity in the unknown material.

Molecular Modeling

Simulations were performed using the Material Studio™ molecular modeling environment—version 5.5 update-2 (Biovia, San Diego, CA). A grid of 0.25 Å was used to compute the unit cell volume data and to generate the computationally dehydrated crystal structure of LEVO. Using DICVOL06 and N-treor in PDXL2 (Rigaku Corporation, Austin, TX) software, powder patterns were indexed using 20 low angle peaks to obtain the crystal system and cell parameters of the unit cell. Checkcell software was used to refine the cell parameters and, knowing the chirality, obtain the space group. Initial crystallographic information files (CIF) were generated using the refined cell parameters, crystal system, space groups and estimated atoms in the unit cell. Materials Analysis using diffraction software (MAUD) was used to estimate the structure of LEVO (dehydrated crystal hydrate) by describing the experimental pattern collected. The refined crystal structure with an R-factor < 10.0% is considered acceptable (52).

Sample Treatment to Study Chemical Degradation of LEVO

LEVO (approximately 5 mg) in duplicate were weighed in open 20-mL glass vials and placed at different storage conditions created in desiccators. The storage conditions were RT/0% oxygen, 60 °C/0% oxygen, RT/21% oxygen, 60 °C/21% oxygen, RT/0% RH, 60 °C/0% RH, RT/75%RH, 60 °C/75% RH. LEVO control samples (approximately 5 mg) were weighed in duplicate, transferred to glass vials, sealed using lids with Polyseal® cone liners and stored at approximately 2 °C. Oxygen concentration was measured using an oxygen gas monitor (Model: Pac 7000, Draeger Safety Inc., Sugarland, TX).

High-Performance Liquid Chromatography

The potency of the samples at six time points was tested by HPLC over 1 month. A Dionex Ultimate 3000 HPLC (Thermo Fisher Scientific, Waltham, MA) equipped with degasser, quaternary pump, automatic injector, column oven, and a UV detector was used for potency determination. Data was collected and analyzed using Chromeleon 7.1 software (Waltham, MA). The separation conditions were based on the literature (53) and modified slightly. At each time point, duplicate control samples and duplicate samples from each of the eight storage conditions were prepared for high-performance liquid chromatography (RP-HPLC) analysis. The diluent for all samples and calibration standards was a solution of 0.01 M sodium hydroxide in methanol. The diluent was prepared by diluting 1.0 mL of aqueous 1.0 M NaOH in methanol to a final volume of 100 mL. Dionex Acclaim® 120 analytical column with C8 stationary phase (250 mm length × 4.6 mm internal diameter; 5 μm particles; column temperature—30 °C) was used for the analysis. The mobile phase was 0.025% trifluoroacetic acid in water (MP-A) and acetonitrile (MP-B). Baseline separation for levothyroxine and all impurities tested was achieved with gradient conditions of 80% MP-A to 0% MP-A over 25 min with a total run time of 33 min. The analysis was carried out at a flow rate of 1 mL/min, sample injection volume—20 μL and analytical wavelength of 223 nm. The method was calibrated and validated at a concentration level of 25, 50, 80, 100, and 120 μg/mL. A sample solution of 100 μg/mL was used quantitative determination of percent LEVO present in the treated sample. The percent LEVO remaining was calculated, according to the following equation:

$$\%LEVO\ remaining = \frac{peak\ area\ of\ the\ sample}{peak\ area\ of\ the\ standard} \times 100\% \quad (1)$$

RESULTS AND DISCUSSION

Thermal Characterization of LEVO upon Exposure to Varying Humidity and Temperature

LEVO (as received) was re-crystallized using methanol and verified for its crystalline purity by comparing the experimental peak positions with the simulated powder diffraction data obtained from single crystal structure data (QQQETG02) deposited in Cambridge structural databased. LEVO was analyzed using TGA. The plot of % weight *versus* temperature (Fig. 1a) shows a loss of 10.5%. Karl Fisher titrimetry was carried out on the LEVO to confirm the weight loss is water. DSC of the sample to characterize the energetics of the loss is shown in Fig. 1a. The water molecules in the crystal hydrate are an integral part of the crystal lattice. So thermal analysis will show the enthalpy associated with the water in the system. The dehydration of LEVO occurs in two steps as seen in Fig. 1a, presumably due to two binding energies, each corresponding to a different energetic environment in the crystal lattice. Katrusiak *et al.* (20) also reported that the two water molecules are independent and not in coordination with Na cations, which forms the crystallographic (001) planes. The DSC thermogram of LEVO showed two endothermic peaks at 92 °C and 117 °C and one exothermic peak at 200 °C. The weight loss from the TGA experiment is consistent with two water molecules being associated with first endotherm and three water molecules associated with the second endotherm.

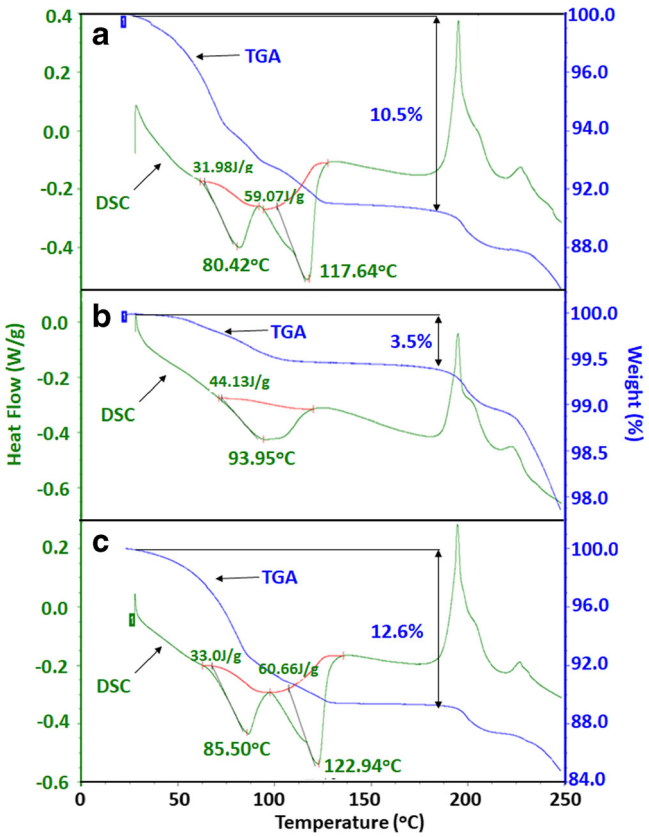

Fig. 1. **a** DSC and TGA of levothyroxine sodium pentahydrate. **b** DSC and TGA of levothyroxine sodium pentahydrate after exposure to 4–6% RH at RT for 12 days. **c** DSC and TGA of levothyroxine sodium pentahydrate after exposure to 100% RH at RT for 12 days

The ratio of enthalpy of vaporization is 4:5, which shows that the later three water molecules are approximately 25% more energetically bound in the crystal system. To determine if the endotherms in the DSC were associated with water loss or degradation, LEVO was heated to 130 °C in the DSC with a nitrogen purge and later analyzed on HPLC. There was neither degradation in terms of assay content nor the presence of any extra peaks. Therefore, the two endothermic peaks observed in Fig. 1a were associated with loss of water only and not decomposition. The decomposition of the LEVO does not begin until 180 °C in the DSC, which is observed as the exothermic peak in the DSC thermogram (Fig. 1a). The molecular weight of LEVO is 888.9 g/mol, if all the five water molecules are removed from LEVO, the molecular weight of LEVO anhydrous drops to 798.9 g/mol, corresponding to a theoretical weight loss of 10.1% on dry basis.

Analysis of the DSC thermogram of LEVO upon exposure to 4–6% RH ($LEVO_{DH}$) (Fig. 1b), shows a single endothermic peak at 93.95 °C as compared to two endothermic peaks in LEVO (Fig. 1a). LEVO upon exposure to 100% RH (Fig. 1c), shows these two endothermic peaks persist. Based on these data, it is clear that LEVO exposed to 100% RH ($LEVO_H$) remains hydrated, while dehydration occurs upon exposure to 4–6% RH.

Further, TGA analysis of the sample stored at RT/0% RH showed a loss of 3.5% of initial weight (Fig. 1b), and the sample stored at RT/100% RH lost 12.6% (Fig. 1c) resulting in the net gain of 2.1% in addition to the 10.5% of moisture lost from initial pentahydrate form. The samples stored at 100% RH remained fully hydrated and added water adventitiously. Thus, a trend has been established that shows the loss of water, as expected, depends on the environmental relative humidity. Each water molecule removed from LEVO yields a loss of approximately 2%; therefore, the sample held at RT/0% RH contained less than two waters.

Characterization of Percent Water Content in LEVO upon Exposure to Different Humidity and Temperature Conditions Using Karl Fisher Titrimetry

Karl Fisher titrimetry was used to compare and verify the results obtained from TGA analysis. The moisture content of the LEVO as received materials was determined to be between 10.32 and 10.92%. The moisture content of the $LEVO_H$ was between 13.12 and 15.37%, in which 10.5% water content was contributed by initial pentahydrate form resulting in a net weight gain of 2.62–4.87%. The moisture content for $LEVO_{DH}$ was found to be between 1.14 and 2.03%. The moisture contents obtained from KF titrimetry are consistent with the observations in the TGA analysis that water in excess of the initial is adventitiously associated.

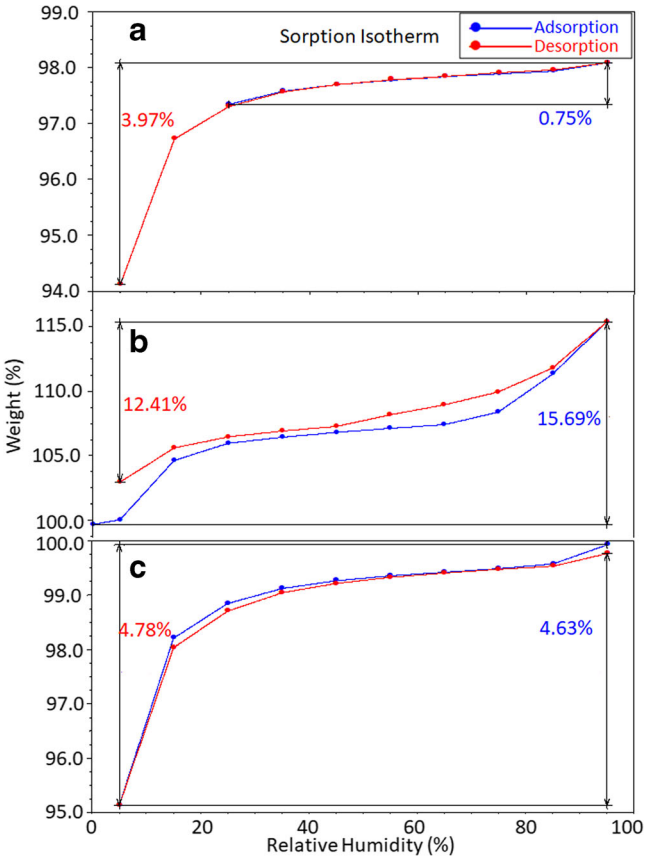

Fig. 2. **a** Water vapor sorption isotherm of levothyroxine sodium pentahydrate powder at 25 °C over the RH range 5–95%. **b** Water vapor sorption isotherm of levothyroxine sodium pentahydrate powder exposed to 4–6% RH for 12 days ($LEVO_{DH}$) at 25 °C over the RH range 5–95%. **c** Water vapor sorption isotherm of levothyroxine sodium powder exposed to 100 % RH for 12 days ($LEVO_H$) at 25 °C over the RH range 5–95%

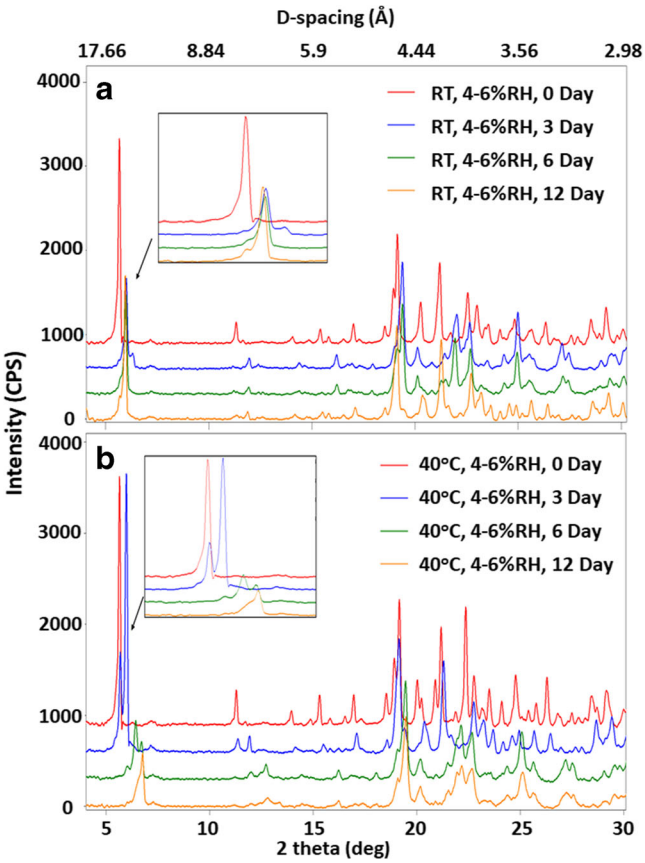

Fig. 3. **a** X-ray diffraction data of levothyroxine sodium pentahydrate crystals after storage at 4–6% RH for 12 days at RT (0, 3, 6, 12 days—top to bottom). **b** X-ray diffraction data of levothyroxine sodium pentahydrate crystals after storage at 4–6% for 12 days at 40 °C (0, 3, 6, 12 days—top to bottom)

Characterization of the Moisture Sorption Response of LEVO upon Exposure Different Humidities

It was reported that LEVO increased in weight by approximately 0.3–0.5% due to moisture absorption/adsorption at 60–70% RH and increased by 0.7% at a relative humidity of 90% (6).

As reported earlier, (7,54), the current moisture sorption studies also show the weight gain of LEVO (as received material) as 0.75%, over the initial approximately 10.5% at 95% RH (Fig. 2a).

The sample was a white-colored solid powder at the beginning of the sorption experiment which converted to a light brown color suggesting degradation, which was confirmed by HPLC to be a 7.54% loss of potency. Figure 2b shows the sorption-desorption isotherms for LEVO to be slightly hysteretic after being held at 4–6% RH for 12 days. $LEVO_{DH}$ shows an increase in weight of 15.69% exposed to 5–95% RH during sorption, while $LEVO_{DH}$ desorption from 95–5% RH lost 13% of total weight gained at 95% RH (Fig. 2b). The sorption-desorption isotherm for a sample stored at 100% RH ($LEVO_H$) (Fig. 2c), shows the loss/gain of water occurring almost reversibly. $LEVO_H$ lost 4.78% weight from 95–5% RH and gained 4.63% weight from 5–95% RH. $LEVO_{DH}$ isotherms were slightly hysteretic. This is consistent with the persistence of the crystal hydrate packing motif under all the conditions studied.

From the above data, it is estimated that LEVO has a critical RH between 40 and 50%. The equilibrium moisture content (EMC) was calculated to be 45.01% based on substitution of the experimental data in the equations below.

$$P = \frac{\left[W \times \frac{A}{100}\right] \pm B}{W - \left[W \times \frac{A}{100}\right]} \times 100 \quad (2)$$

$$\mathrm{EMC} = \frac{P}{P + 100} \times 100 \quad (3)$$

where W is initial sample weight, A is initial percentage moisture, B is weight change at equilibrium, and P is percent moisture on a dry basis.

In summary, the reports (7,8) show degradation of LEVO at low humidity, which combined with the current study, demonstrates that the crystal hydrate undergoes dehydration at % RH values below 45.01%.

Characterization of the Crystal Structures of LEVO Using Powder X-ray Diffraction Methods

The results from moisture sorption and associated energetics show the changes in the hydration states of the LEVO. It was, therefore, necessary to elucidate the associated structural changes under different humidity and temperature conditions to fully understand the physical behavior. Changes in the crystal structure during storage at RT and 40 °C at 4–6% RH and 100% RH, were monitored with PXRD at 0, 3, 6, and 12 days (Figs. 3a, b and 4a, b). Upon exposure to 4–6% RH, Fig. 3a, b, show that the position of the peak at $2\theta = 5.67°$ (001) plane shifted to higher angles with a time of storage, *i.e.* 6.02 °2θ at day 12. Concurrent with the shift in the peak position, the peak area is reduced by a factor of 8. Also, the peak at $2\theta = 11.32°$ shifted to 11.39°, which corresponds to a reduction in the direction of (1, − 1, 0) plane. The above changes in the PXRD pattern lead to a reduction in the volume of the crystal lattice. In terms of d-spacing, the change at $2\theta = 5.67$ is 0.88 Å, whereas at higher angles the relative changes in d-spacing are minimal (< 0.1 Å). Conversely, upon exposure to 100% RH at different temperatures (Fig. 4a, b), there was no change in the peak position as well as d-spacing at $2\theta = 5.67$ and 11.52° which shows LEVO's crystal structure persists at higher humidity. The studies under each condition were continued up to 30 days; however, the PXRD patterns were relatively constant after 12 days.

The changes in the PXRD pattern (Fig. 3b) at 4–6% RH and 40 °C indicate a progressive change of phase, but as is shown later from the structure solution from powder data of the dehydrated material, the packing motif persists consistently with Fig. 3a. The decrease in relative intensity and broadening of the peaks in the PXRD pattern with increasing time of storage at low humidity reflect a gradual but significant increase in disorder.

Figure 5a shows eight unit cells of LEVO with the (001) plane emphasized and Fig. 5b shows LEVO (computationally dehydrated) with the same (001) plane emphasized. Figure 5a shows the (001) planes are associated closely with the water molecules (shown in van der Waal's radii) and are in accordance with Katrusiak *et al*'s report (20). As the water molecules leave the crystal lattice, the surviving structure is that of a dehydrated-hydrate according to Morris *et al.* (55). This is followed by chemical degradation under normal conditions. It was shown earlier that water molecules form an integral part of the crystal lattice forming/stabilizing the lattice channels *via* interaction with the oxygen atoms of the

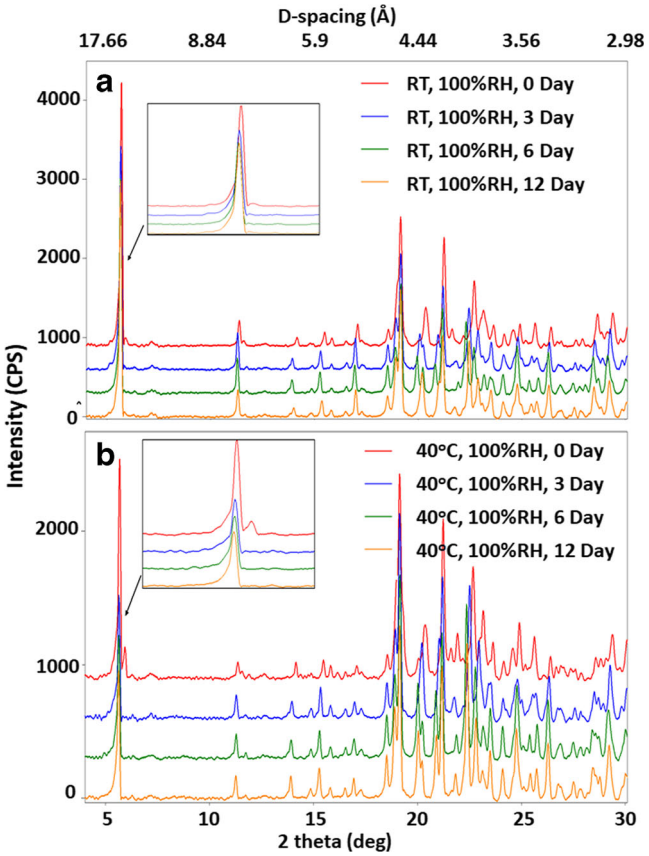

Fig. 4. **a** X-ray diffraction data of levothyroxine sodium pentahydrate crystals after storage at 100% RH for 12 days at RT (0, 3, 6, 12 days—top to bottom). **b** X-ray diffraction data of levothyroxine sodium pentahydrate crystals after storage at 100% RH for 12 days at 40 °C (0, 3, 6, 12 days—top to bottom)

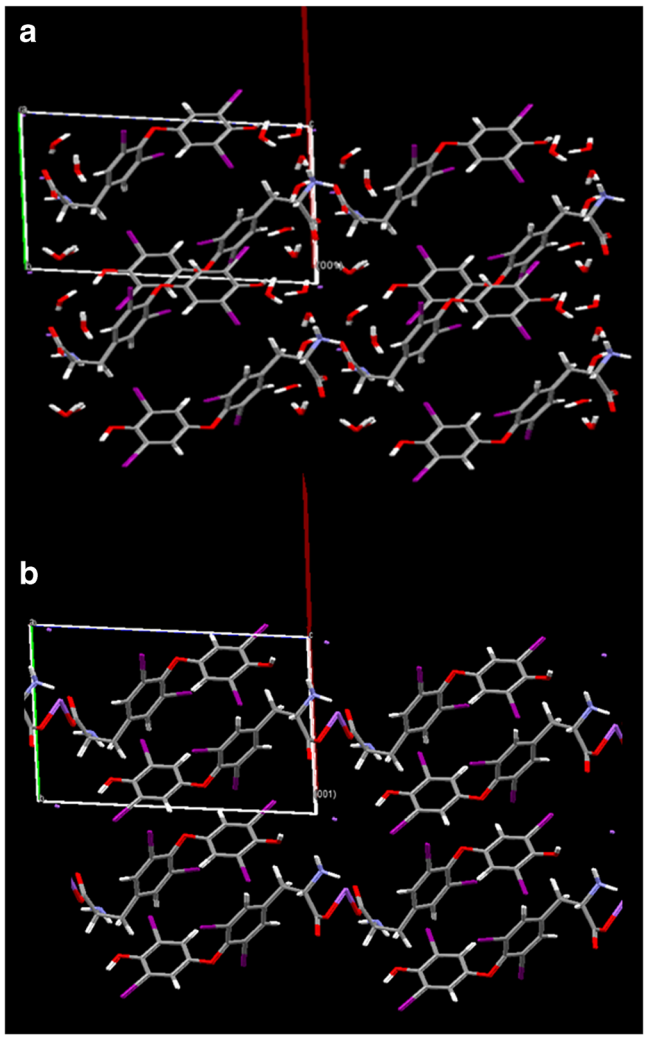

Fig. 5. **a** Crystal structure of levothyroxine sodium pentahydrate with 001 plane. **b** Crystal structure of levothyroxine sodium (computationally dehydrated) with 001 plane

terminal 4-hydroxy diiodophenoxy ring *via* hydrogen bonds and electrostatically with sodium ions. The peak shifts of observed in the PXRD patterns of LEVO are consistent with an intermediate and partially disordered, dehydrated crystalline phase of LEVO and the subsequent degradation. This intermediate crystalline phase is relatively unstable and using the experimental pattern of the dehydrated crystal hydrate, is indexed in the crystal structure solution section.

To further characterize the changes in crystal structure observed at low humidity and high-temperature conditions, LEVO was analyzed using a complementary technique, vertical transmission variable temperature X-ray diffraction, to confirm the changes previously observed at low humidity. A temperature range of 30 to 130 °C was selected based on the TGA/DSC analysis which showed a complete loss of water at 130 °C in the earlier thermal analysis section. The PXRD data were collected in cycles from 30 to 130 °C with a temperature ramp of 10 °C per scan allowing the sample to equilibrate at the desired temperature for 15 min before collecting the PXRD pattern. Figure 6a shows the overlay of the PXRD pattern obtained from vertical transmission X-ray analysis.

As seen in Fig. 6a, the 001 peaks at $2\theta = 5.67°$ shifts to higher angles when heated from 30 to 130 °C. The crystalline phase converts to non-crystalline phase at 130 °C. Figure 7

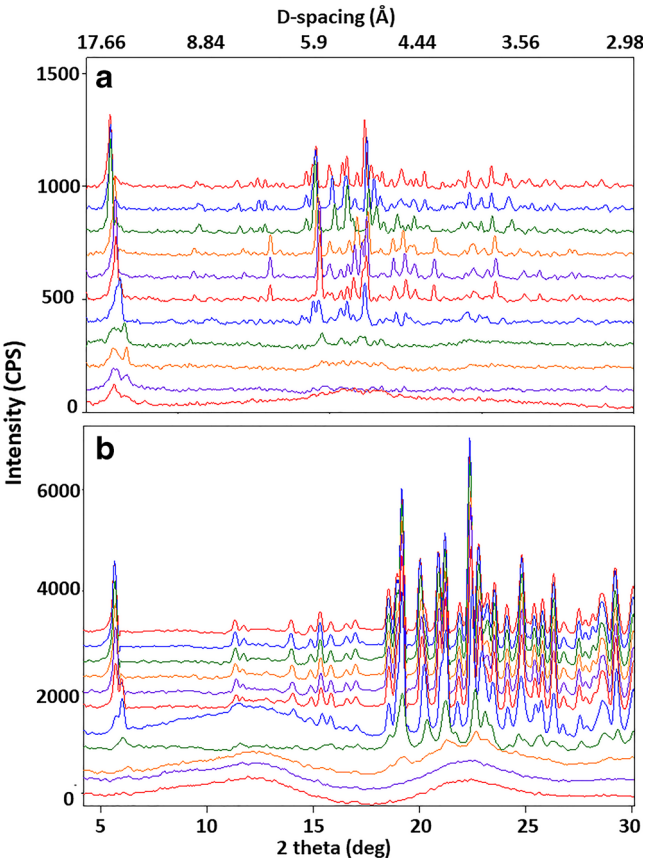

Fig. 6. **a** Levothyroxine sodium pentahydrate—variable temperature data collected in vertical transmission using linkam stage. (30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 °C—top to bottom). **b**. Levothyroxine sodium pentahydrate—variable temperature data collected in BB reflection using hot plate. (30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 °C—top to bottom)

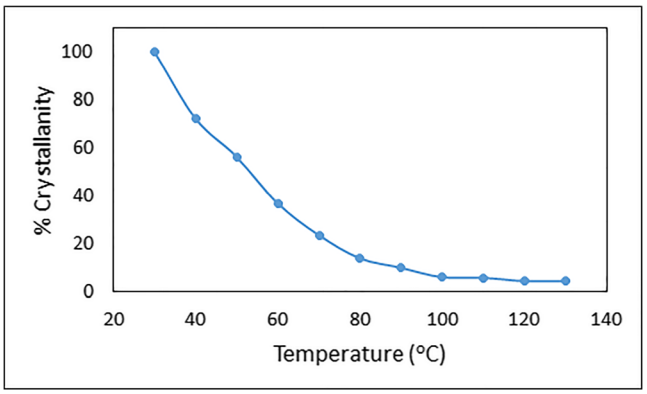

Fig. 7. % crystallinity of levothyroxine sodium—variable temperature data collected in vertical transmission using linkam stage (30–130 °C)

shows the percentage crystallinity calculation of LEVO from 30 to 130 °C. The peak intensities obtained from vertical transmission were relatively low for powder indexing purposes. Therefore, comparable experiments were performed in reflection (Bragg Brentano) mode (Fig. 6b). It was observed that the unit cell of the crystal hydrate contracted in the direction of 001 crystallographic plane. PXRD fingerprint between 10 and 40° 2θ did not change significantly consistent with a persistence of the packing motif until 90 °C. These observations are also consistent with observations previously discussed from the PXRD measurements at RT and 40 °C at low humidity conditions. Upon complete removal of water at 130 °C, the material is disordered.

The Crystal Structure Solution from PXRD of the Dehydrated Crystal Hydrate of LEVO

The powder X-ray diffraction data of partially disordered, crystalline phase of LEVO (dehydrated crystal hydrate) obtained at 110 °C was indexed using Dicvol (56) and N-treor (57) to obtain the crystal system and cell parameters. The crystal system at 110 °C is triclinic as is that of the pentahydrate. Checkcell (58) was used to obtain the space group which was P1, and refine the cell parameters and assign peak position to each plane in the crystal structure. Materials Analysis Using Diffraction (MAUD) software was used to generate the crystal information file (CIF) by refining the cell parameters, micro-strain and refining the backgrounds. The crystal structure obtained can be seen in Fig. 8. The final parameters obtained are compared with original pentahydrate structure in Table I, which shows a reduction in the cell parameter a, c, α, β, and γ, and increase in the value of b. Thus, the unit cell contracts in a and c directions, while expands in b direction. The reduction in cell parameter c in the direction of (001) crystallographic planes matches with the observation from the LEVO to low humidity and high-temperature conditions. One water molecule corresponds to the volume of 20–30 Å (59). LEVO contains five water molecules, which corresponds to the volume of 100–150 Å. The volume of LEVO reported by Katrusiak *et al.* was 1220.07 $Å^3$ (20), loss of five water molecules should yield a volume of 1120 $Å^3$. The unit cell volume of the new crystal structure of the dehydrated form is 1114.53 $Å^3$, which matches with the theoretically expected cell volume. The

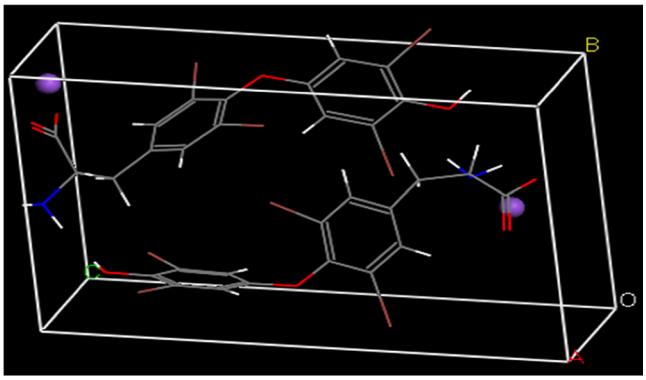

Fig. 8. Crystal structure of dehydrated crystal hydrate of levothyroxine sodium obtained from MAUD software

packing motif of the LEVO's dehydrated crystal hydrate is same as that of pentahydrate form.

HPLC Analysis to Determine Percentage Loss of LEVO upon Exposure to Different Humidity, Temperature Conditions, and Presence of Oxygen

As the structural changes of LEVO have been established using DSC, TGA, moisture sorption, and PXRD methods, it is important to study, how/if the physical instabilities precede the chemical instability. LEVO was exposed to different combinations of oxygen, relative humidity, and temperature to study their role in chemical degradation of LEVO. The conditions were chosen based on realistic storage condition and in accordance with ICH guidelines. Since the main goal of the HPLC studies was to determine percentage loss of LEVO at different humidities and oxygen levels, a higher temperature was chosen to provide the least amount of stress yet cause a measurable amount of degradation. A previous study (26) showed that the solid-state LEVO heated at 50 °C for 30 days under ambient conditions would remain relatively stable, but would degrade at higher temperatures (60, 70, and 80 °C). Therefore, 60 °C was chosen as the higher temperature for the HPLC studies.

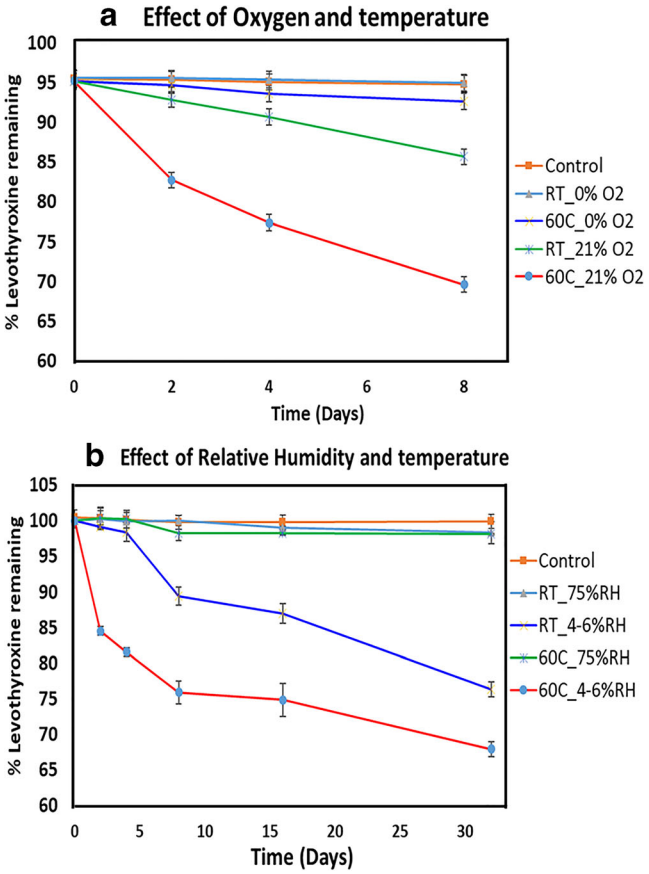

Fig. 9. **a** Effect of oxygen and temperature on levothyroxine sodium pentahydrate. **b** Effect of relative humidity and temperature on levothyroxine sodium pentahydrate

Figure 9a shows the plot of percent LEVO remaining as a function of time for samples held on each of the stations. The amount of LEVO in the control samples and the samples stored at RT/0% O_2 remained relatively stability with or without oxygen at RT, as long as they remain hydrated. The amount of LEVO decreased by 3.1% at 60 °C/0% O_2, indicating the effect of temperature. However, the amount of LEVO decreased by 10.6% at RT/21% O_2

Table I. Crystal Structure Data of Levothyroxine Sodium Pentahydrate Crystals *versus* Levothyroxine Sodium Pentahydrate at 110 °C

	Original crystal structure of levothyroxine sodium pentahydrate	Levothyroxine sodium pentahydrate at 110 °C
Crystal system	Triclinic	Triclinic
Space group	P1	P1
Unit cell dimensions	$a = 8.2489$ Å	$a = 7.663$ Å
	$b = 9.4868$ Å	$b = 9.868$ Å
	$c = 15.8298$ Å	$c = 15.542$ Å
	$\alpha = 84.1387°$	$\alpha = 79.25°$
	$\beta = 83.1560°$	$\beta = 75.5°$
	$\gamma = 85.0482°$	$\gamma = 83.11°$
Volume	1220.07 Å ^{3}	1114.53 Å ^{3}
Z	2	2
R factor	0.0408	0.0825
2-theta range for collection	4–40	4–40
Index ranges	$4 < h,k,1 < 20$	$4 < h,k,1 < 20$

and by 26.3% at 60 °C/21% O_2 showing that even at RT, $LEVO_{DH}$ degrades rapidly once it is subjected to molecular oxygen.

Figure 9b shows the plot of the amount of LEVO remaining as a function of time for samples held at different humidity and temperature conditions. The amount of LEVO remaining in the control samples and the samples stored at RT/75% RH and 60 °C/75% RH remained relatively constant, while the amount of LEVO for samples stored at RT/4–6% RH and 60 °C/4–6% RH decreased rapidly. At the end 32 days, the amount of LEVO remaining in the sample stored at RT/4–6% RH decreased by 23.75% and 60 °C/4–6% RH decreased by 33.1%. The data demonstrates that LEVO degrades when it is dehydrated and remains stable as long as it is hydrated even in the presence of molecular oxygen. In the presence of molecular oxygen, higher temperature, and low humidity conditions the variability in the assay results also increases. Thus, it can be concluded that the LEVO starts to degrade as soon as the water molecules leave the crystal structure and allow the entry of oxygen and oxidation.

CONCLUSIONS

LEVO tablets are the most recalled drug product in the history of USFDA. The crystal hydrate may be dehydrated due to the use of hygroscopic excipients and/or high mechanical stress induced by the different processes. The findings reported here demonstrate the root cause of the sub-potency issues, failed dissolution, excipient led degradation, and degradation of the LEVO during the manufacturing processes.

For the first time, the changes in the crystal structure of LEVO were elucidated leading to the conclusion that LEVO does indeed degrade due to the loss of water molecules in lattice channels allowing access to molecular oxygen without a change in the packing motif and introducing varying degrees of disorder. The waters of hydration play a vital role in the stability of crystal hydrate as shown from the diffraction and thermal analyses. The outcomes support the hypothesis that the chemical stability of LEVO lies in the integrity of the crystal hydrate structure, which when disturbed by several possible mechanisms leads to the degradation of LEVO. In addition to this, the extremely low concentration of LEVO (0.018–0.21%) in tablets makes it practically impossible to elucidate these phenomena by examination only of the final drug product.

Building new prior knowledge for LEVO, by using the data from the current study and solid-state decompositions of crystal hydrates from previous findings, will help to better understand the stability problems associated with LEVO in the drug product. Using new prior knowledge, LEVO tablets can be moved from most recalled drug products to least recalled drug products benefitting 1.5 million patients in the USA. New or generic drug product development for LEVO can build quality within the product right from the product development stage, production stage, packaging, and storage to ensure that the LEVO retains itself as crystal hydrate (with five water molecules) throughout the shelf life.

In general, drug substances are prone to degradation in presence of water. ICH guidelines suggest the accelerated stability conditions of 40 °C/75% RH as stress-inducing studies. However, this work shows that LEVO is an exception, *i.e.*, remaining stable at higher humidity and is unstable at low humidity conditions. Therefore, accelerated stress condition of including expected ranges of exposure should be added to the guidelines of crystal hydrates as a part of new prior knowledge. This would help identify different types of instability associated with crystal hydrates during the development stage, and also form the basis for establishing pharmaceutically equivalent drug products. Using the "new prior knowledge" approach, such products may be developed more rapidly with a firm scientific rationale that may aid in approval and controlling costs.

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