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Dry powder inhaler: influence of humidity on topology and adhesion studied by AFM

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Abstract

In the dry powder inhalers (DPIs), the adhesion results of the interactions between the active substance and the excipient. The carrier and the micronized drug particle morphologies are believed to affect the delivery of the drug. In this work, the couple studied was the lactose monohydrate and micronized zanamivir, used for the treatment of influenza. In a first approach, observations by scanning electron microscopy (SEM) have shown that the relative humidity (RH) greatly influenced the zanamivir amount fixed on the lactose monohydrate surface. This paper deals with the direct measurement in controlled atmosphere by atomic force microscopy (AFM) of the forces and the interaction ranges between a zanamivir probe and a lactose substrate. Selected zanamivir crystals were attached to the standard AFM probe. Different RH have been used in order to determine influent parameters permitting to identify the nature of adhesion forces between them. This study demonstrated that the increase of RH modified progressively the surface topology of the two components and increased the adhesion force.

Keywords: Dry powder inhaler; Adhesion force; Atomic force microscopy; Relative humidity

1. Introduction

Dry powder inhalers (DPIs) are believed to be the most suitable alternatives to metered dose inhalers (MDIs) for inhalation therapy (Timsina et al., 1994). Many applications of DPIs have been tried for asthma, chronic bronchitis, emphysema, cystic fibrosis and pulmonary infections (Smith and Bernstein, 1996).

Dry powder inhaler formulations consist of interactive mixtures. In these mixtures, an adhesion occurs mainly between a micronized powder component and a coarse grade excipient. Homogeneity of the mixture is improved by this physical interaction.

In the present study, the carrier particles (monohydrate lactose) have to follow three crite-

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ria: improvement of flowability of drug particles into inhalation device (capsule) during filling process, increase in dispersing property of cohesive drug particle during emission, and diluent of drug on lower dosing (Kawashima et al., 1998). Numerous studies have reported improvements of the technique including the used of smoother surface of lactose particle (Ganderton, 1992), the reduction of the particle size of the carrier (French et al., 1996; Steckel and Müller, 1997) and the use of ternary materials in the powder formulation during delivery from DPIs, in term of adhesion (Staniforth, 1996). By other techniques, several authors (Lucas et al., 1998; Zeng et al., 2000) have observed that the addition of a small amount of fine lactose to the blend improved the fine particle fraction (FPF) of micronized drug in the lung.

To our knowledge, no quantitative investigations of interfacial forces between a drug and its carrier have been conducted according to the relative humidity (RH). The primary adhesion forces for a dry uncharged particle on a dry uncharged substrate consist of Van der Waals and electrostatic forces. In humid environments capillarity condensation gives rise to a very large capillarity force increasing the total adhesion force. When the RH is above 50%, the capillarity force dominates (Finot et al., 1996). The main technique to determine the adhesion force between micronized drug particles and carrier substrate was the centrifuge technique (Podczeck, 1997; Busnaina and Elsawy, 1998).

Our objective in this article is to observe the effect of the water adsorption on the surface reactivity of the drug and carrier particles and the consequences on their adhesion.

This present study deals with the ability of atomic force microscopy (AFM) to determine surface evolution and adhesion forces between a micronized drug particles and the lactose monohydrate in various RH. The measurement of adhesion force by AFM previously was performed on various mineral surfaces such as gypsum, calcite, mica, graphite (Finot et al., 1999, 2000; Lesko et al., 2001) or on a single polymer sphere (Biggs and Spinks, 1998). Therefore, it was not still applied to pharmaceutical materials like lactose monohydrate and micronized zanamivir (Von Itzstein et al., 1993; Chapple et al., 2000). Zanamivir is the first in a new class of drugs to be developed for the treatment of influenza.

The AFM results will compare with the observations by SEM of zanamivir/lactose blends at various RH in order to demonstrate the relevance of this technique in terms of measurement of the adhesion forces.

2. Materials and methods

2.1. Materials

Zanamivir micronized particles were used as supplied by GlaxoWellcome, Inc. The distribution of particle size of micronized zanamivir was an unimodal distribution with a geometric volume mean diameter of 2.5 μm and a geometric standard deviation of 1.5 (Laser scattering, particle suspension in methanol, Coulter LS130, Coultronics, USA). These micronized particles will be completely dissolved during the crystallization process.

Spray-dried lactose was supplied by GlaxoWellcome (HMS, The Netherlands). Lactose particles have presented an unimodal volume diameter distribution with a geometric volume mean diameter typically of 150 μm and a geometric standard deviation of 1.8.

All materials were stored at ambient conditions: 25 °C, 32% of RH.

2.2. Sample preparation

For SEM studies, three zanamivir and lactose monohydrate blends (20:80 w/w) were prepared using a Turbula mixer (model T2C, Glen Mills Inc., Maywood, NJ, USA) during 10 min.

In order to obtain a reference plane, zanamivir was crystallized at room temperature (25 °C) in bi-distilled water (Milli-Q water, 18.3 MΩ cm, obtained from a Nanopure UV, Barnstead Station, UK). Zanamivir crystals consist of platelet shaped crystals 5–200 μm long, confirmed by SEM microphotographs. In order to minimize the roughness, the lactose was compacted in manual

mode with an instrumented single punch tablet machine (Korsh EK0, Berlin, Germany) equipped with 10 mm flat-faced punches, at an applied pressure of 100 ± 25 Mpa. Tabletting will inevitably alter the surface morphology of the lactose (i.e. brittle material). For this study, the compact constituted a plane reference at submicronic scale (few μm^2 area) independently of the cracks observed at larger scale.

Each sample was placed at three values of RH (0, 32 and 85% RH). Thermodynamic equilibrium was reached by placing the blends, the compacts of lactose and the zanamivir crystal probes into three pre-equilibrated desiccators in RH. Their RH was adjusted by P_2O_5 for 0% RH, and by sursaturated salt solution $MgCl_2$ for 32% RH, and KCl for 85% RH at 25 °C (Norm of the French agency of normalization AFNOR number NFX 15014). According to water adsorption kinetics previously realized on the materials to establish the equilibrium time, the zanamivir crystals were removed after 2 days, and the lactose compacts or the blends after 21 days.

2.3. Methods

X-Ray diffraction was performed on lactose and zanamivir in order to provide an indication of the variation of crystalline/amorphous content and polymorphism induced by the storage. X-ray diffraction is performed using CPS 120 INEL diffraction system fitted with a localization curve-Cu-anti cathode (120°, 4096 channels).

The photomicrographs of sample of the three blends sputtered with nickel were taken using scanning electron microscopy (SEM, JEOL 6400F, Japan).

AFM measurements were performed using a commercial atomic force microscope (Nanoscope IIIa, Digital Instruments, Veeco Inst., Santa Barbara, CA). All images were acquired in air using contact mode AFM (contact-AFM) or oscillating contact mode (Tapping Mode™ AFM; TM-AFM) with the E-type scanner (12 μm) and the J-type scanner (150 μm). For contact mode AFM imaging, V-shaped silicon nitride cantilevers with a nominal spring constant of $k = 0.01–0.06$ N/m (Park Scientific Instruments, Sunnyvale, CA) were used. The TM-AFM experiments were performed using silicon cantilevers of spring constant $k =$ 20–25 N/m (Digital Instruments, Santa Barbara, CA). In order to remove contaminants, the tips were exposed to ultra-violet (UV)–ozone for 10 min, which permits the removal of the hydrocarbons. For each tip used, the sensitivity response was determined from amplitude calibration plots on glass cover slips. By measuring the resonant frequency of the different cantilevers (with or without fixed particle), the associated spring constant k was calculated using the expression (Cleveland et al., 1993): $k = 2(\pi L v)^3 w(\rho^3/E)^{1/2}$. L and w represent the length and width, respectively, of the cantilevers as supplied by the manufacturer, v the resonant frequency, E the Young's modulus, and ρ the density of material of the cantilever.

For topographical investigations, the radius of curvature of the sharpened AFM tip was estimated to 10–20 nm on calibrated grid samples. Zanamivir crystal samples were prepared by sprinkling the crystalline powder onto double-sided adhesive tape. Concerning the lactose, the compact was fixed with a sticky tab onto the AFM sample stub.

Force measurements have been carried out with silicon nitride cantilever (200 μm long) with a spring constant $k = 0.1–1$ N/m. Selected zanamivir single crystal (with a size range of 10–100 μm long axis) was glued using epoxy glue at the free end of the AFM cantilever, which had an inclination of 12°. Some of zanamivir probes after sputtered with nickel were observed by SEM. Elemental composition analysis were carried out by energy dispersive spectrophotometry (EDS-Oxford-Inca Energy Software). The electron beam energy was 20 keV. Force measurements were performed in a cyclical manner (extending and retracting of sample). The frequency of the sample movement was fixed at 0.5 Hz (i.e. about 50 nm/s) in such way that the effects of viscosity were avoided (Burnham et al., 1993; Hao et al., 1991). The contact time between particles was estimated to about 1 s. The deflection of the cantilever (perpendicular to the sample surface) due to the force produced by the sample was recorded using both AFM system and an independent recorder (DSP Lock-in amplifier,

model SR 850, Stanford Research System, CA). Forces versus separation curves were calculated from the sensitivity and the calculated spring constant of the cantilever.

All AFM studies were carried out under controlled atmosphere. The commercial atomic force microscope was able to normally perform with a humidity relative range of 0–90%. The drying process (0% RH) was assured by a flux of helium gas. The RH of 32 and 85% was achieved by passing decarbonated nitrogen through distilled water. The humidity was controlled and measured with an accuracy of 1% in placing a hygrometer (Quick 74880 Novo Ebro Gmbh, Germany) and the AFM head under a glass bell jar. The temperature under the bell jar was 25 °C.

One set of measurements has always been carried out with the same type of cantilever in order to guarantee a comparative analysis of forces. To reduce the measurement uncertainties, a statistic analysis from 3 to 10 cycles of measurements has been performed in 10 different sample locations. Ten zanamivir probes have been utilized facing 3 different lactose compacts for each humidity surrounding.

3. Results and discussion

Particles adhesion in DPIs is known to be a complex problem. The powder blends observation is the first approach to confirm the affinity between the drug and the excipient. In environmental conditions, it is important to study the eventual modification of global structural characterization of the samples. Surface forces convey nothing without the knowledge of the surface reactivity. As a result, correlation with computed force values and topographical and structural becomes essential.

3.1. SEM observations

The surface topography of blends of zanamivir and lactose (20:80 w/w) stored, respectively at 0 and 85% RH were obtained by SEM (Fig. 1). In any cases, the blend is a binary ordered mixture but the RH greatly influenced the zanamivir amount fixed on the lactose surfaces. Thus, the more RH increases, the more zanamivir particles adhere.

3.2. X-Ray analysis

The X-ray spectrums of zanamivir shown no modification of phase composition according to the variation of RH (Fig. 2a). α-monohydrate lactose is the major constituent (> 99%) of lactose studied (Fig. 2b). For 0 and 32% RH, a trace of anhydrous lactose was detected and which disappeared at 85% RH. In any case, concentration of amorphous phase was too low to be quantifiable.

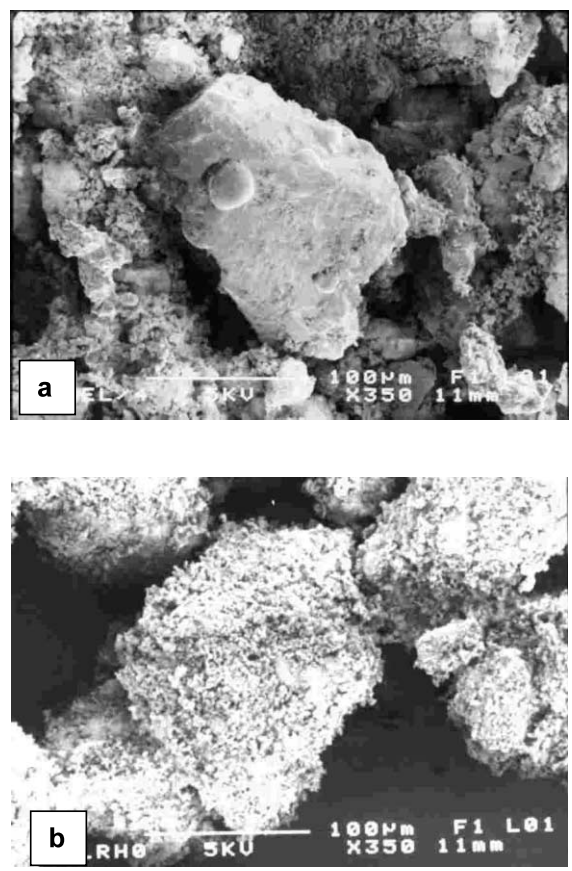

Fig. 1. SEM images (× 350) from the blend of lactose monohydrate and micronized zanamivir stored at 0% RH (a) and 85% RH (b) until reach thermodynamic equilibrium at 25 °C.

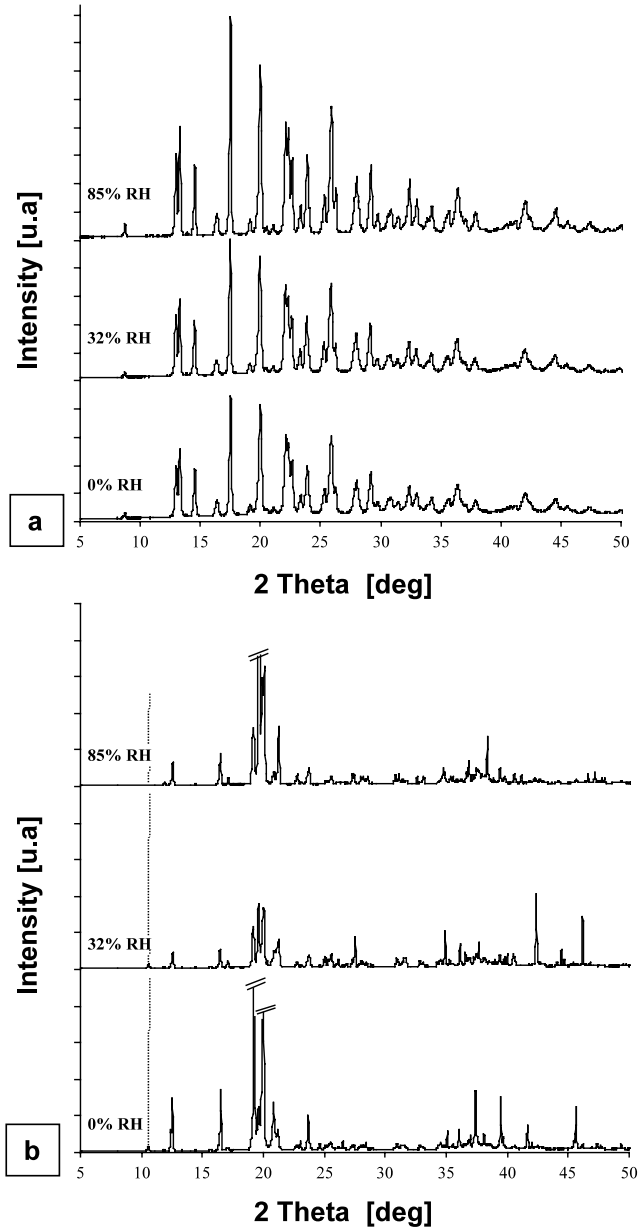

Fig. 2. X ray spectrum (a) zanamivir and (b) lactose, stored at 0, 32 and 85% RH. On lactose spectrum, the dashed line represents the peak of anhydrous lactose disappearing at 85% RH.

3.3. Study of surfaces reactivity

It is obvious that the interaction is correlated to the surface roughness and the contact area between zanamivir and lactose. In order to analyze only the influence of the RH on the adhesion force, the surface roughness was minimized by recristallization of the drug particles.

The different AFM techniques (friction in contact-AFM, phase imaging in TM-AFM) were per-

formed to image the eventual evolution of the morphology and contamination of the surfaces (i.e. differences in chemical constitution). For the surfaces evaluation, analysis of images obtained after each sample preparation was carried out. No evident modification of the nature (i.e. crystal phase composition) of the material on the surface was observed in friction and viscoelasticity imaging.

The lactose compact surfaces were sensitive to the variation of RH (Fig. 3). The increase of RH induced significantly the coalescence of lactose entities. The morphology of lactose particles was quantified by a combination of different descriptors, derived from the length along the longer axis (L) and the half-height (H). To complete the analysis, the length (L) over half-height (H) ratio was calculated. The average particles length (L) was 169.40 ± 21.94 nm and the half-height (H) was 49.81 ± 13.97 nm at 0% RH. These values increased to 591.80 ± 56.66 nm for the length and to 88.03 ± 26.92 nm for the half-height at 85% RH. The L/H ratios were, respectively 3.66 ± 0.94 at 0% RH, 7.21 ± 1.71 at 32% RH, 7.49 ± 1.72 at 85% RH. The average roughness (R_a) has been measured. This roughness is given by the expres-

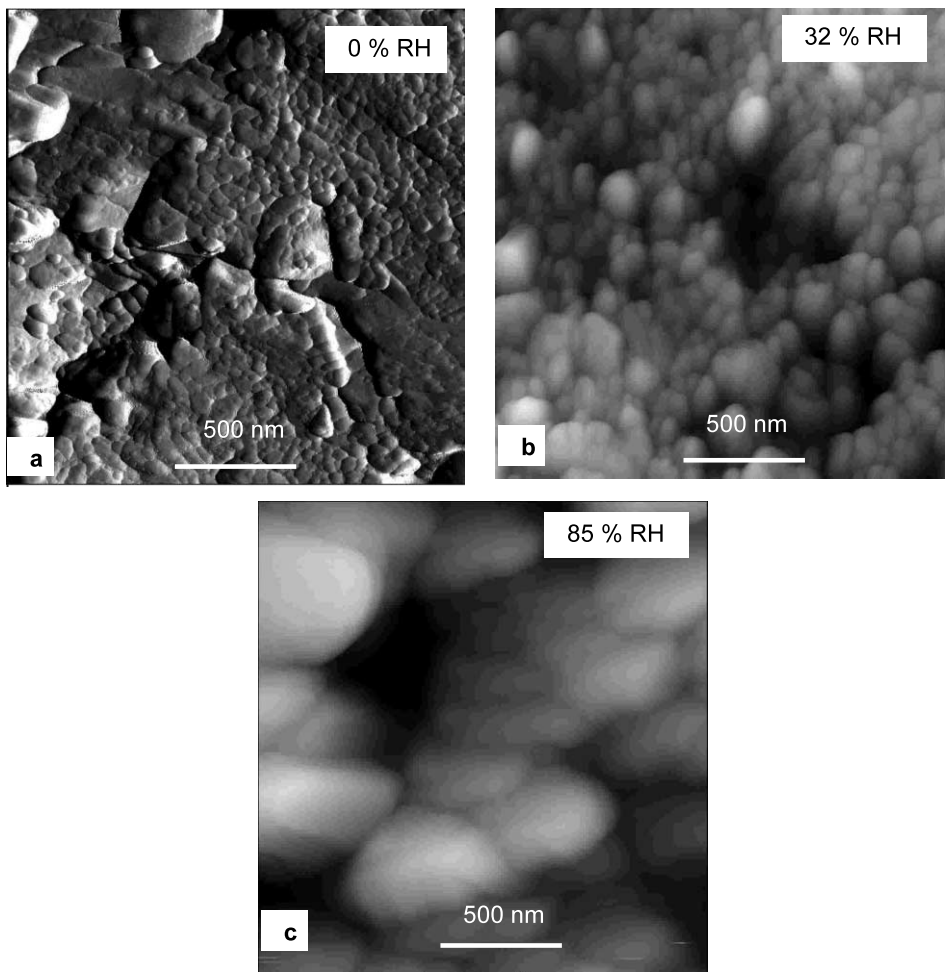

Fig. 3. TM-AFM height images (2×2 μm^2) from the surface of compact lactose in air. (a) RH = 0%; Relative height = 250 nm; Mean roughness = 37.29 ± 14.79 nm. (b) RH = 32%; Relative height = 150 nm; Mean roughness = 25.95 ± 6.08 nm. (c) RH = 85%; Relative height = 250 nm; Mean roughness = 23.41 ± 5.65 nm.

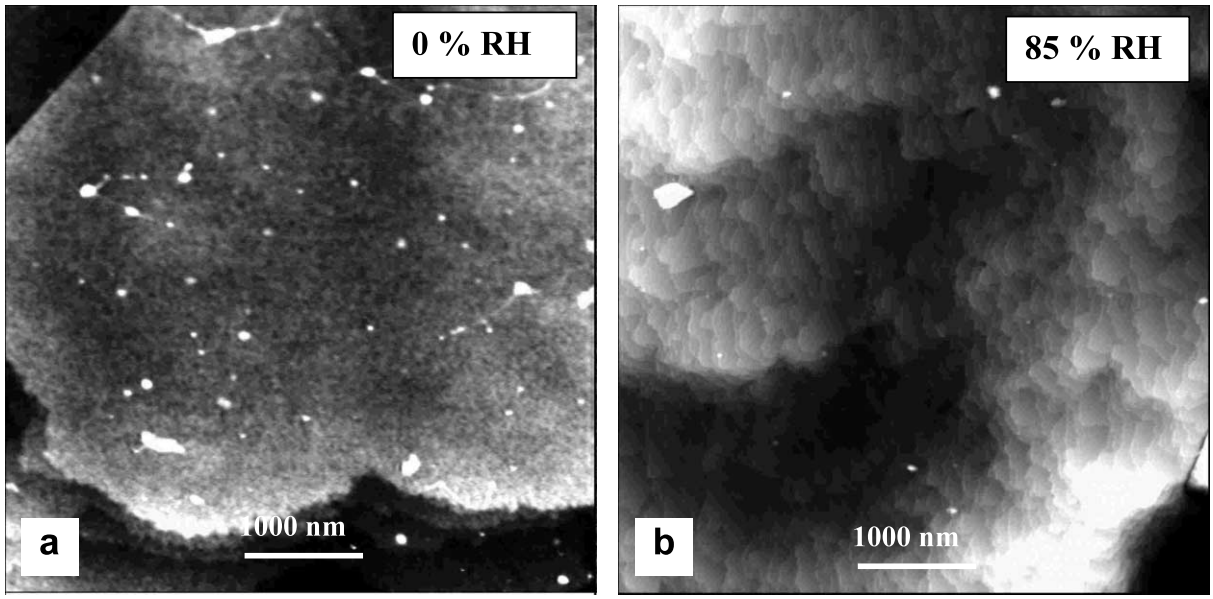

Fig. 4. AFM images of zanamivir crystal (5×5 μm^2). (a) RH = 0%; Relative height = 5 nm; atomically flat terraces as large as 25 μm^2, separated by steps can be seen. (b) RH = 85%; Relative height = 25 nm; the (001) surface extends over 1 μm^2. In both cases, the height of the terraces is about 0.9 nm.

sion: $R_a = \Sigma_i |Z_i|/N$ where N is the number of points of the image (512×512 points). This represents the arithmetic average of the absolute values of the surface height deviations measured from the mean plane. Roughness statistics were performed on images of 1×1 μm^2 sized. The values of the total surface roughness (R_a) were 37.29 ± 14.79 and 23.41 ± 5.65 nm at 0 and 85% RH, respectively.

From RH values 32% onwards, we have formation of few layers of water adsorbed on the surface. The microstructure is modified by a dissolution-precipitation process induced by the presence of water (Finot et al., 1997). The number of aggregate decreases whereas the average particle size increases. The lactose particles shrink and raise the local supersaturation in the water layer adsorbed. Larger entities shrink at lower rate. Smallest entities are completely dissolved and contributed to the expense of the larger entities. This mechanism is called 'Ostwald ripening' (Ostwald, 1896).

Observations on zanamivir crystals were sum up in the Fig. 4. At 0% RH, the AFM images of the surface of zanamivir crystals revealed more extended terraces than were usually observed at higher RH. The average terrace width was 503.80 ± 182.23 nm at 0% RH. These values decreased to 176.26 ± 15.66 nm at 85% RH. The increase of the RH induced a reduction of the width of the terraces. This difference could be explained by the increase of the particles mobility induced by the presence of adsorbed water on the surface of the crystal at high RH. Thus, the zanamivir particles were able to migrate in any directions. However, the heights of the terraces were a few multiples of the (001) lattices spacing of zanamivir. The height of the steps between two terraces was 0.89 ± 0.02 nm.

The perfect crystallization of zanamivir has allowed to consider it as probe (Fig. 5). As shown by SEM observations (Fig. 5b), zanamivir probe is representative of the size. In the same time, EDS analysis realized on zanamivir probes in the three areas indicated in the figure, shown that the glue epoxy (containing sulphure S) was not present on the crystal free surface in contact with lactose (Fig. 5c).

Contrary to zanamivir crystals, the roughness of the lactose particles and their poor mechanical

cohesion have led us to use the lactose as substrate.

3.4. Measurements of zanamivir/lactose interaction

Forces, liable to appear in air, are Van der Waals force, capillary force, and long-range electrostatic force. No long-range electrostatic forces were measured whatever the RH. The interaction range was restricted to few nanometers. Interaction analysis indicated a dependence on both contact area and spring constant k of the cantilever.

Interaction measurements were performed with usual calibration process to transform experimental cantilever deflection curves as a function of the vertical scanner displacement Δz to force-distance curves. Using the slope of the retraction deflection

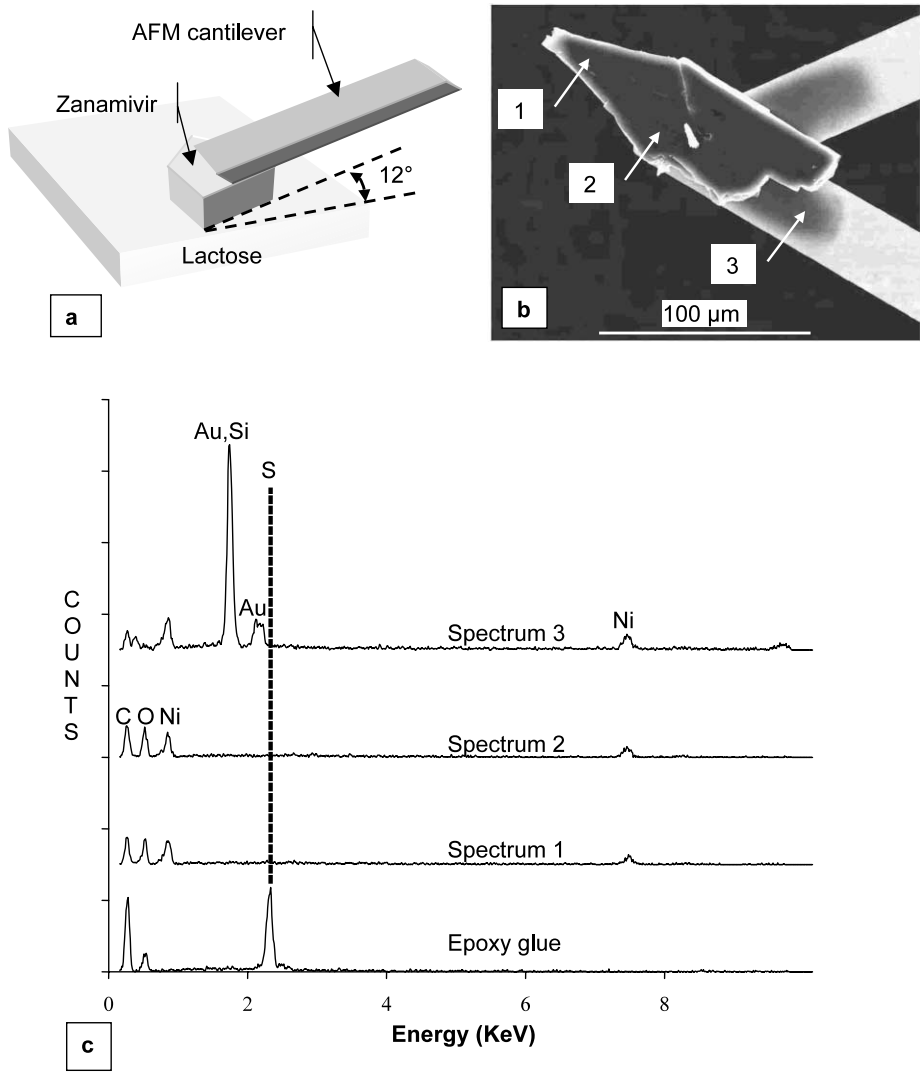

Fig. 5. (a) Cross section of the contact between the zanamivir crystal and the compact lactose. (b) SEM image of zanamivir crystal fixed at the free end of a V-shaped AFM cantilever (bar: 100 μm). (c) Elemental composition analysis EDS of the three areas indicated on figure b. The S peak relative to the epoxy glue composition (dashed line) is not present on the zanamivir surface in contact with lactose.

curves in the contact region, the cantilever deflection is then converted into a force F using the Hooke's law: $F = k_{exp}\ \Delta z$, where k_{exp} represents the measured spring constant of the cantilever. From deflection versus probe-sample separation, using a MATLAB program (The Mathworks, Inc., CA), the force versus separation curve was determined. The Fig. 6 showed an example of force calculations for zanamivir/Lactose, respectively at 32 and 85% RH. The zero force was defined as the force when the tip retracted from the surface. The retract force curve showed an important hysteresis due to the presence of water layer. The capillary force increases the pull out force. In order to quantify contact forces normal to the surface in air, a strong spring constant was chosen (0.1–1 N/m) to avoid all lever torsions at the approach of the surface. Beside, cantilevers with a high spring constant allows us to estimate the high value of the gradients and thus to achieve solid–solid contact.

Adhesions between zanamivir crystals and lactose were first measured at 0% RH. At 0% RH, only the contribution of van der Waals forces could be considered. Van der Waals force depends on the nature of materials and the geometry of the couple. The results are summarized in the Fig. 7. The effect of the contact number between the two components was put in evidence. For instance, an average value of 233.35 nN with a standard deviation of 37.44 nN was measured. This adhesion could reach 750 nN. The measurements showed a dispersion of about 71%. In fact, the dispersion is due to the number of allowed contacts between the carrier particles and the drug crystal, which depends specifically on the roughness of the carrier. This high variability could not permit us to obtain the intrinsic adhesion force value. By considering the cumulative distribution, the median value has been considered. The median value was 180 nN. Adhesion did not evolve in dry air and remained to their minimums $F_{min} = 70$ nN.

The same experiments were performed at 32 and 85% RH. At 32% RH, forces strongly increased. This contribution results from the formation of a water meniscus between the probe and the substrate. This capillary force called Laplace's force can be therefore valued in subtracting the measured values from the van der Waals force given for 0% RH (Finot et al., 1996). Hence, for the same RH (32%), this Laplace's force reached 75 nN. An increase of adhesion was significant at 32 and 85% RH. The adhesion at 85% RH are reported in the third curve of the Fig. 7. The quasi-constant difference of 290 nN compared to median forces measured at 0% RH is ascribed to the capillary force, which seems to be identical, whatever type of forces. The minimum adhesion value for each curve was considered the true value corresponding to the minimum contact area. From 0 to 32% RH and from 32 to 85% RH, the adhesion values are multiplied by two at each step.

The contact area is actually limited by the surface roughness of carrier and therefore the effective area engaged in the contact is constant considering the AFM cantilever inclination (12°). The invariability of the contribution of these forces for tested probes is significant of the surface roughness. This roughness limits also the contact area and plays an important role in the adhesion rising when the angle between surface lattices varies. The Laplace's force being above all a geometrical force, the constant capillary force (deviation of 55 nN) suggests that the contact area between crystals is still the same whatever the orientation of faces in contact.

4. Conclusion

The forces measurement by AFM demonstrated that the increase of RH increased the adhesion force between them according to the following hierarchy:

$$F_{0\%\ RH} < F_{32\%\ RH} < F_{85\%\ RH}$$

These results are relevant with the SEM observations of the blends zanamivir/lactose.

At high RH (above 32%), diffusion process on the lactose surface may be bring about the formation of grains in new phase. X-ray analysis was not allowed to shown modification of the phase composition induced by the humidity conditions of storage. It was not excluded a modification of the crystalline/amorphous content.

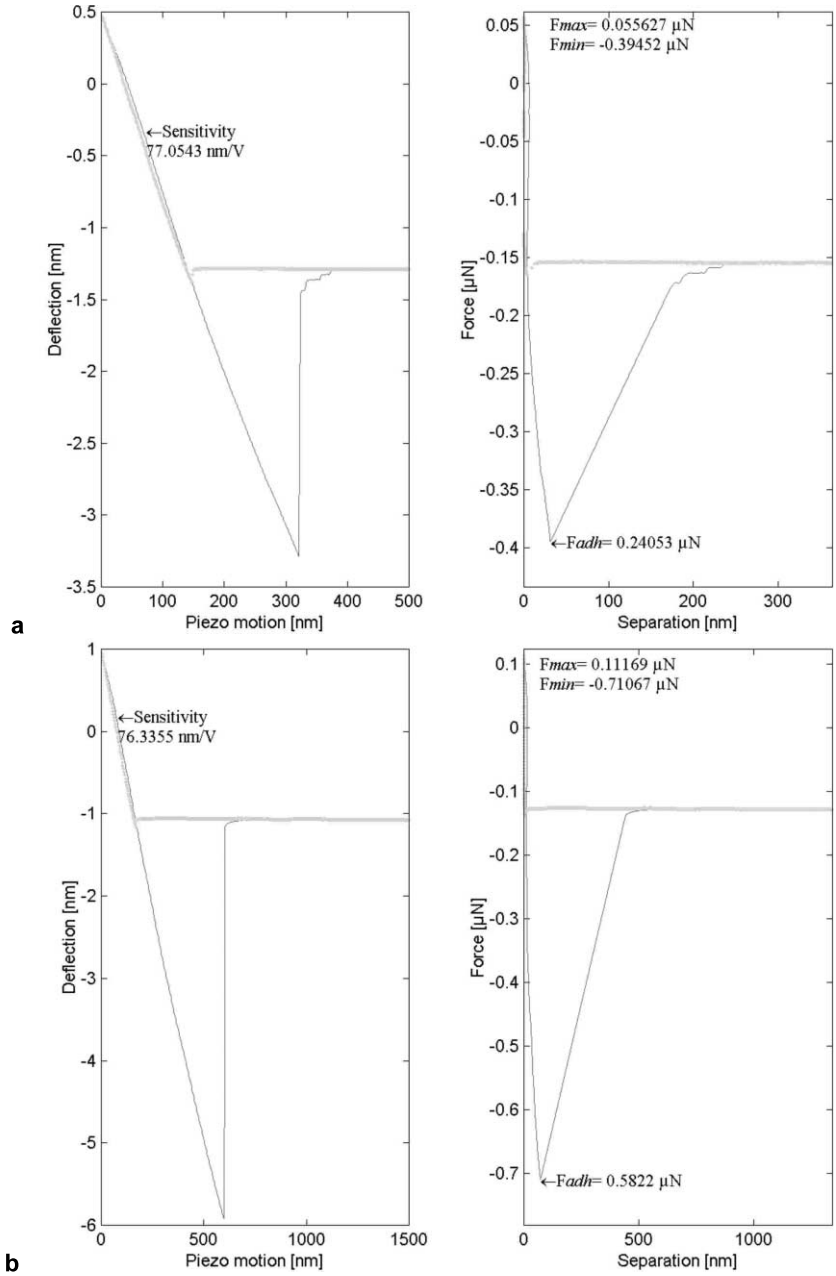

Fig. 6. Illustrations of the measurement of deflection of the cantilever versus the extension of the piezoelectric tube (on left) and the corresponding calculated force versus separation curve (on right) obtained at 32% RH (a) and 85% RH (b) with the same sensitive cantilever ($k = 0.12$ N/m). The bold lines (approach) show the motion of the sample (Lactose) towards the probe (zanamivir), the solid lines (retract) from the probe. The calculated adhesion force was 240 nN at 32% RH and 582 nN at 85% RH.

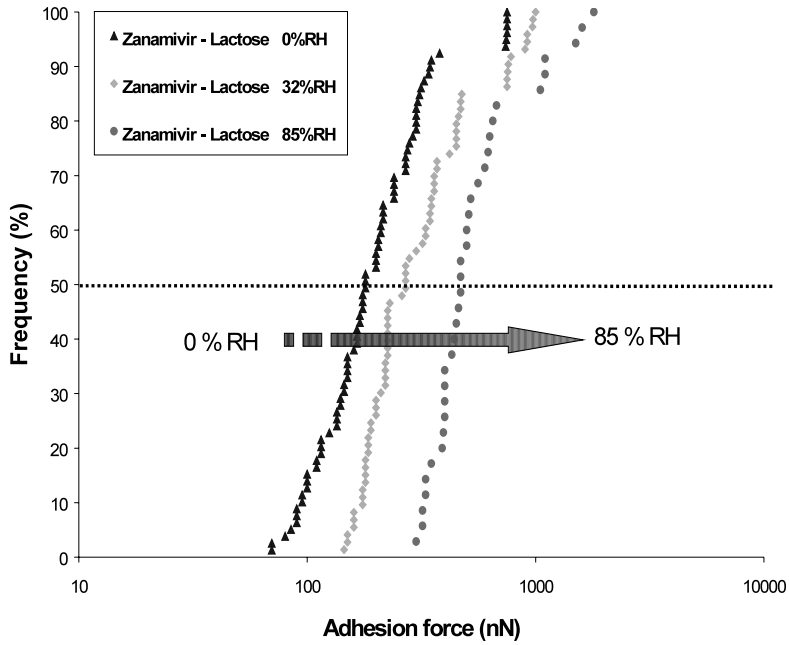

Fig. 7. Adhesion measured by AFM between the zanamivir crystal and the compact lactose. This graph represents the cumulative of experimental measurement expressed in percentage for each RH: 0, 32 and 85% RH.

The AFM technique permits not only the adhesion forces measurement between carrier and drug. This method could provide useful information on surface reactivity induced by the water sorption.

Acknowledgements

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