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Topological Structure and Chemical Composition of Inner Surfaces of Borosilicate Vials

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ABSTRACT: The use of atomic force microscopy (AFM) and x-ray photoelectron spectroscopy (XPS) is described to characterize the inner surfaces of pharmaceutical vials. The two type I borosilicate glasses included in this study slightly differ in their amounts of alkaline oxides. The topography and chemistry of the inner surfaces of vials are predominantly caused by the forming process. A structural and chemical modification of the inner surface of vials was also observed when exposing the surface to different pH conditions and special treatment like washing and sterilization, which are routine operation steps during galenical manufacturing.

KEYWORDS: Atomic force microscopy (AFM), borosilicate glass, topography, x-ray photoelectron spectroscopy (XPS), vials

Introduction

Drug interaction with the primary packaging material is a common problem in pharmaceutical research and development. The pharmaceutical industry in particular is challenged with this issue because most of the sensitive new biotechnological products for parenteral use are stored in glass containers, for example, vials and syringes. The *United States Pharmacopeia* and the *European Pharmacopeia* specify two types of glasses suitable for parenteral use, type I and type II (*1*). However, for highly resistant type I borosilicate glass, a variety of different formulations exist. In terms of surface properties, variability is even found within one single lot of glass containers.

The inner surface properties of glass containers are not simply determined by the composition of glass; they are also greatly influenced by the forming process (*2, 3*). Consequently, the *European Pharmacopeia* requires a surface test in addition to a crushed-glass test for the characterization of glass containers for pharmaceutical use. This hydrolytic resistance test provides important information about the durability of inner glass surfaces against water attack. Chemical components of the glass matrix that are leached out by aqueous solutions can cause different reactions with the incorporated drug, such as precipitation, aggregation, and oxidation of drug molecules. Further, loss of protein in the solution due to adsorption to glass container walls is a well-known phenomenon (*4, 5*). One can imagine that adsorption of drugs to the glass walls of pharmaceutical vials is especially problematic at low drug concentrations.

In the present study the influence of the forming and washing process on the structure of the inner surface of vials to be used for pharmaceutical products is investigated. In general, type I vials are formed by heat from tubing glasses. Prior to filling, the vials are routinely washed and sterilised, that is, depyrogenated, with dry heat. The two types of type I borosilicate glasses used in this investigation differed with regard to their physico-chemical properties and, especially, with regard to their thermal expansion coefficient.

Atomic force microscopy (AFM) is a powerful tool to image insulating surfaces such as glass on a nanometer scale and to measure the size of tiny structures in three dimensions (*6*). Topographic features, for example, lenses, remaining particles, craters and holes, might be

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created during technical processing of glass tubes and vials, as shown in the following studies. The lateral resolution achieved in these investigations was about 10 nm, whereas the vertical resolution was less than 1 nm. AFM allows investigators to measure forces in the piconewton range, and at the same time it enables study of biological specimens *in situ* in a physiological environment, which might become interesting for drug interactions in solution with primary packaging containers. AFM has also been used to measure the extent of protein adhesion on different glass surfaces (*7*). The cited study showed that a slight variability in hydrolytic resistance of the glass surface can result in a significantly different adhesion behavior.

In order to determine the chemical element distribution on different glass surfaces before and after chemical and physical treatment, x-ray photoelectron spectroscopy (XPS) was used (*8*). The combination of topological and chemical information provided unique insights into surface processes.

Materials and Methods

In these studies two kinds of type I borosilicate glasses containing different amounts of oxide components were compared. The samples from FIOLAX® glass (Schott, Mainz, Germany) contain a slightly higher percentage of sodium, aluminum, and calcium oxide than the samples from KG-33® glass (Kimble, Vineland, NJ, USA). Accordingly, the mole fraction of silicon and boron oxide is lower in FIOLAX than in KG-33.

All AFM measurements were performed in air and at room temperature on a TOPOMETRIX Explorer (Thermomicroscopes, Sunnyvale, CA, USA). The silicone cantilevers with integrated silicone tips (Pointprobe Nanosensors, Wetzlar, Germany) used for noncontact mode measurements had force constants of about 42 N/m and resonant frequencies of about 300 kHz. Images were taken with a resolution of 500 × 500 pixels. The scanning rate was at 2–3 lines per second. All figures presented are unfiltered images. Horizontal or vertical leveling was applied by the Topometrix SPMlab software, version 4.0, which can cause a horizontal or vertical shadow if the imaged features are high relative to the surrounding area. Topography images are shown with the corresponding height scale.

For investigation of the inside of tubing glasses and vials, the samples were carefully broken and cleaned in a dry nitrogen stream (purity: 99.9997%) in order to eliminate glass dust. The samples were glued to the sample holder of the AFM instrument in such a way that the area to be measured at the edge of a glass piece was in the measuring plane. Special vial treatments such as washing and sterilization were conducted before breaking the glass samples. Two water-washing cycles were performed under pressure at about 60 °C. Then the vials were passed through a tunnel of about 300 °C for sterilization/depyrogenation.

The effects of exposure to acidic and basic solutions were investigated as follows. FIOLAX and KG-33 vials were filled with aqueous solutions, adjusted to pH 3, 5, 7, and 10 with the addition of HCl or NaOH, and kept at room temperature for 3 days before emptying and rinsing with 5 mL purified water. The surfaces were then dried in a stream of nitrogen and prepared as described above.

Chemical analysis was performed by XPS (PHI 5400, ESCAlab, Perkin Elmer Corporation, Baton Rouge, LA, USA) in ultra-high vacuum. While fixing the samples onto the sample holder, one had to make sure that the sides of the crooked glass pieces did not hinder the analyzing electron beam.

Results

Tubing glass

Figures 1-A and 1-C show 5-μm images of the inside of untreated FIOLAX and KG-33 tubing glasses, respectively, that are used for vial production. Typically, particles of up to 25 nm in height, equally distributed over the surface, were found on FIOLAX tubing glass, but not on KG-33 tubing glass. However, as seen in Figure 1-B, these particle structures were easily rinsed off by water. Figure 1-B and 1-D are 5-μm images taken of the inner surfaces of FIOLAX and KG-33 tubing glass, respectively, after being rinsed and dried in pure nitrogen shortly before the measurement. After rinsing, the topography and roughness of both surfaces were very similar. Particle structures, like the ones on FIOLAX glass, were soluble in water and swept away by either the AFM tip or by gently rubbing the surface with a cloth. Similar observations have been described by other authors who investigated glass surfaces under atmospheric conditions (*9, 10*).

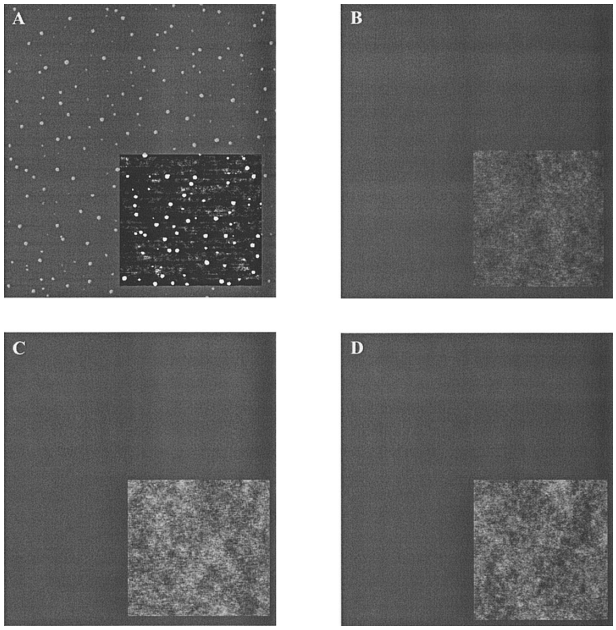

Figure 1

Topography of the inner surfaces of untreated (A, FIOLAX; C, KG-33) and rinsed (B, FIOLAX; D, KG-33) tubing glasses (size: 5 × 5 μm; greyscale: 25 nm). The insets show the corresponding parts of the images at a greyscale of 3 nm.

Vials

For the manufacture of vials from tubing glass, heat is first applied at one end of the vertically or horizontally positioned tubing glass in order to form the neck, then to part and smooth the bottom of the vial. Sections of the inner surface close to the bottom of the finished FIOLAX and KG-33 vials are presented in Figures 2-A and 2-B, respectively. These surfaces were rinsed with water before measuring because particles, like in tubing glass, nucleate and grow on the surface when exposed to the atmosphere.

As seen in Figure 2, the inner surfaces of the vials were dotted with circular, slightly raised, lens-like features of different sizes that measured about 30 nm in height and up to 1 μm or more in diameter. For FIOLAX and KG-33 glass, the frequency and the size of the lenses were at a maximum near the bottom and diminished towards the neck of the vial. These lens-like structures, as shown in Figure 2, remained stable over extended measurement time, not only when measured in air but also in aqueous conditions when the AFM measurement was performed in a fluid chamber (data not shown).

In the case of water-rinsed KG-33 vials, additional ring-like structures were distinguished homogeneously over the entire inner surface of these vials (Fig. 2-B). The average size of a ring feature was around 100 nm in diameter and 3 nm in wall height. Additionally, a cavity in the center of each ring was observed. Under ambient conditions on untreated glass surfaces, particles were positioned on top of the ring features (Fig. 3A). These particles were easily removable with a cotton stick or a cloth or by rinsing the surface with water or acid (Fig. 3B).

Vials after washing and sterilization

A study was conducted to show the effects of washing and depyrogenating vials. As shown in Figure 4, this treatment leads to severe topological effects of the lenses. The lenses found on the surfaces of FIOLAX and KG-33 glass vials before treatment were washed out centrically due to the washing and sterilizing pro-

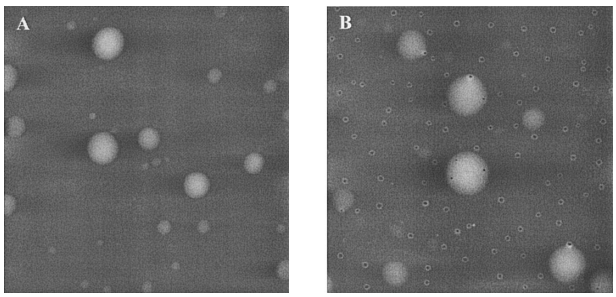

Figure 2

Topography of the inner surfaces of a rinsed (A) FIOLAX and (B) KG-33 vial (size: 5 × 5 μm; greyscale: 38 nm).

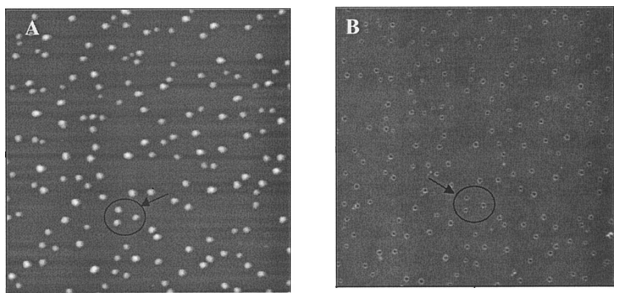

Figure 3

Topography of the inner surfaces of a KG-33 vial, (A) before and (B) after rinsing (size: 5 × 5 μm; greyscale left: 57 nm, right: 10 nm).

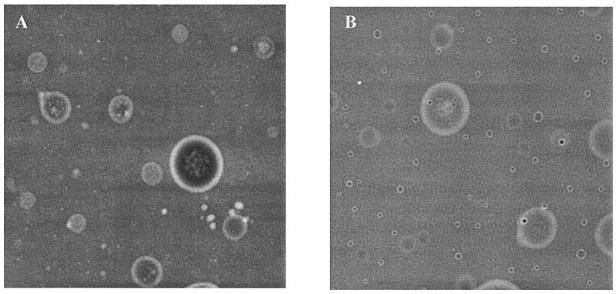

Figure 4

Topography of the inner surfaces of a washed and sterilized (A) FIOLAX and (B) KG-33 vial (size: 5 × 5 μm; greyscale: 30 nm).

cess leaving so-called craters. Typically at this state a small edge was left around each lens (Fig. 4-A and 4-B).

Lens structures under acid and basic conditions

Results for FIOLAX and KG-33 vials after exposure to acidic and basic solutions are shown in Figures 5 and 6, respectively. All images represent areas from the inner surface of the bottom part of the vials, which are relatively rich in lenses.

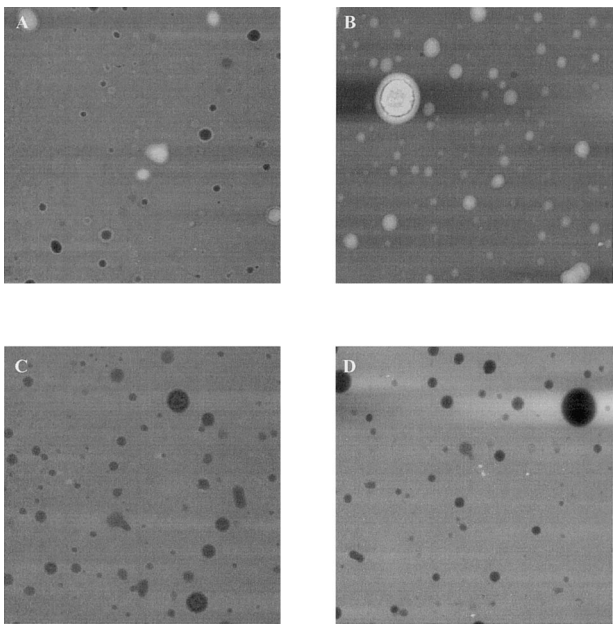

Figure 5

Topography of the inner surfaces of FIOLAX vials stored in aqueous solutions of pH 3 (A), pH 5 (B), pH 7 (C), and pH 10 (D). Size: 10 × 10 μm; greyscale: 54 nm.

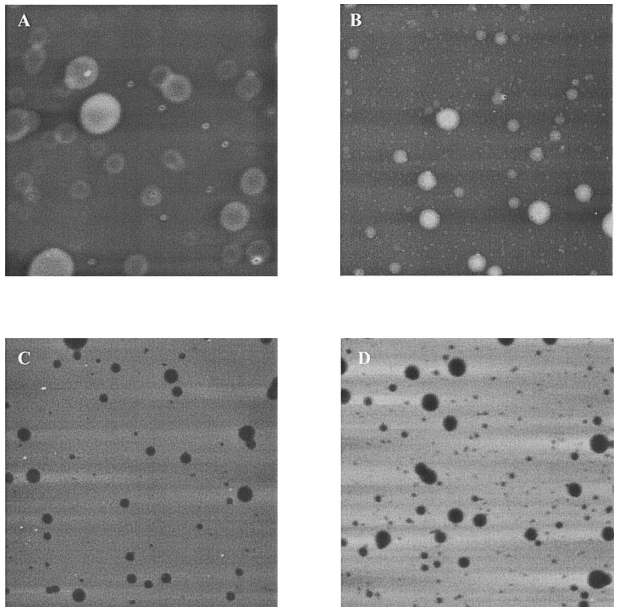

Figure 6

Topography of the inner surfaces of KG-33 vials stored in aqueous solutions of pH 3 (A), pH 5 (B), pH 7 (C), and pH 10 (D). Size: 10 × 10 μm; greyscale: 41 nm.

The images obtained from vials stored at pH 3 (Fig. 5-A and 6-A) were similar to those obtained after water washing and sterilization/depyrogenation. Craters have formed after lenses have been washed out.

In contrast to the treatment with acid (pH 3) after three days (Fig. 5-B and 6-B), the lens structures did not become craters in purified water. On FIOLAX glass, only some of the largest lenses have become craters.

When the test solutions contained NaOH and the pH rose above 7, the lens washed out without leaving edges. Holes were formed instead of craters (Fig. 5-C, D and 6-C, D). Based on the difference in color contrast, the washing process was faster with more alkaline solutions. In alkaline solutions the washing process was faster on KG-33 glass than on FIOLAX glass, whereas in acidic solutions the reverse was observed. These results strongly suggest that lenses on the inner surface of FIOLAX and KG-33 vials vary in oxide content.

Chemical composition of the inner surface of tubing glass and vial

In search of a method to determine the chemical composition of the glass surface, XPS was identified as the

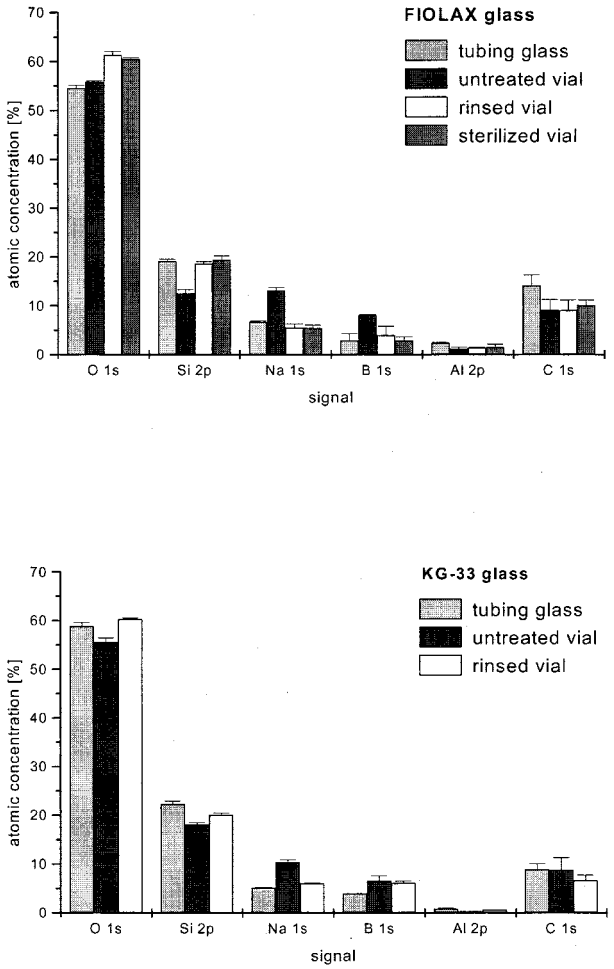

Figure 7

XPS results showing the atomic concentration of elements on inner surfaces of FIOLAX and KG-33 glass. Oxygen, silicium, sodium, boron, aluminum, and carbon signals were recorded from tubing glass (light grey), untreated (black) and rinsed vials (white), and, for FIOLAX only, vials after washing and sterilization (dark grey).

only method available to measure the percentage of oxide components present in the top few atomic layers of the glass surface. Although single structures could not be resolved on the glass surface samples, mean values of the elemental distribution over 100 μm^2 were achieved by XPS. This technique allowed for the investigation of both the enrichment of alkaline oxides on the surface caused by the forming process and the loss of surface elements due to leaching and washing out.

The distributions of the main elements on FIOLAX and KG-33 glass surfaces are seen in Figure 7. Each value represents two measurements on different sample surface locations. Both FIOLAX and KG-33 glass record a significant increase of sodium and boron and a slight decrease of aluminum on the inner surface of vials as compared to the inside of the corresponding tubing glass. Rinsing the vial surface results in a significant decrease of sodium and boron for the FIOLAX glass and, to a lesser degree, a decrease of sodium for the KG-33 glass. A further drop of boron is observed when the vials have passed the washing machine and the tunnel in the pharmaceutical filling line (corresponding data for KG-33 not shown). In other words, after passing the sterilization tunnel the vials almost reach the initial surface composition of tubing glass.

In order to get a surface profile, the glass surface was sputtered with 4 keV Argon for 10 min, and simultaneously the element distribution on a single spot on the surface was monitored. Consequently, the enrichment of sodium in the first 10 to 20 nm of the surface of a FIOLAX glass vial could be observed (Fig. 8). The sudden drop of carbon on the surface is related to the normal atmospheric surface contamination.

Discussion

From a technical point of view, the manufacturing process of both FIOLAX and KG-33 tubing glass is practically the same. However, on the basis of numerous measurements, it was shown that characteristic particle structures could only be found on inner surfaces of FIOLAX tubing glass, but not on the KG-33 type of glass (see Table I). We assume that the presence of particle structures on FIOLAX glass is related to the higher alkali content of the glass surface as compared to KG-33 glass. Temperatures of up to 1200 °C are applied in order to form the vials from the glass tubing. This process causes alkali ions to diffuse to the surface. Consequently, the percentage of alkali is elevated at the surface, favoring the formation of salt crystallites by the reaction with water steam or gaseous acids from air, for example, carbon dioxide. These salty precipitates are soluble in water when not subsequently burned in at higher temperatures in the furnace (*11, 12*).

The same explanation is valid for the particle structures observed on all vial surfaces examined. However, the particles on vial surfaces are no longer a criterion of distinction between FIOLAX and KG-33 glass because all the vial surfaces were locally heated. Consequently, alkali oxides migrated to the surface. Indeed, XPS results indicate that the particle structures

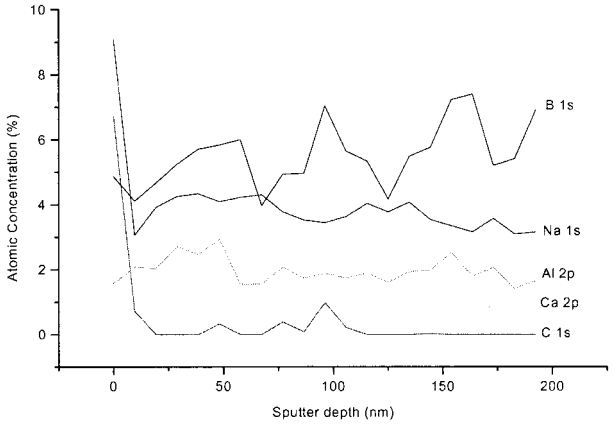

Figure 8

XPS-depth profile of the inner surface of a rinsed FIOLAX vial for boron, sodium, aluminum, calcium and carbon. Sputter rate: 17.6 nm/min.

contain high levels of sodium ions. A significant decrease of sodium on KG-33 vials occurs when the surfaces are rinsed with water, leaving particle-free ring structures. These ring-like structures observed on KG-33 vials are well-known also from other glass types and probably can be attributed to local etch pits, induced by humidity, heat, or chemical reactions from the glass manufacturing process (e.g. dissolution), most probably at glass defects or local inhomogeneities.

On the other hand, the lens structures form instantly during the vial-forming process. Since the parting and smoothing of the bottom of the vial requires temperatures up to 1200°C, volatile components like alkali oxides and boric acid are released (*11*). These compounds recondense on cooler surfaces as droplets or lenses, causing the enrichment of sodium and boron on the surface. The characteristic distribution of the lenses on the inside of vials is caused by the evaporation cone directed towards the container wall. Lenses on FIOLAX glass surfaces are more easily dissolved in water and acid because FIOLAX glass contains more alkali oxides than KG-33 glass. Consequently, the percentage of alkali in those lenses is higher. The higher content of silica dioxide in KG-33 glass contributes to a higher resistance to bases. This is verified because sodium borate is highly soluble in acid, whereas the hydrolysis of the silica network is favored in alkaline conditions (*2, 13*).

In spite of the high solubility of sodium borate and other alkali salts in water, the dissolution of glass compounds, like lenses, is still relatively slow. However, it has to be considered that at the end of the forming process the vials have to pass through high-temperature furnaces at about 600 °C in order to reduce stress in glass walls. At that point, condensation of alkali borate on the surface burns in and becomes glassy and transparent (*11*). The solubility of this condensation film, and therefore of the lenses, is drastically reduced. It should be noted that alkali borate form the simplest glasses!

Another interesting point about the lenses is the forming of craters when attacked by water or acid. It seems that the lenses are not simply covering the surface but react with the glass bulk itself. Because of diffusion processes, the remaining edge is probably rich in silica and therefore less soluble at lower pH. Strong bases remove the surface layer by layer, and they are able to break up the entire silica network of glass-forming holes.

TABLE I
Typical Features on Tubing Glass and Vials

	Tubing Glass	Vials
FIOLAX	■ *Particles* (soluble and removable)	■ *Particles* (soluble and removable) ■ *Lenses* (*craters* are formed in acidic solutions and water; *holes* are formed in neutral and alkaline solutions)
KG-33	None	■ *Particles (soluble and removable)* ■ *Rings* ■ *Lenses* (*craters* are formed in acid and water; *holes* are formed in neutral and alkaline solutions)

The results obtained in this study are in good agreement with extractable results reported for surface analysis of FIOLAX and KG-33 glass vials (*14*). The amount of extracted alkali ions stands in close relation to the hydrolytic resistance of glass surfaces.

Conclusions and Outlook

In current pharmaceutical production of very sensitive active substances, the interaction of the compounds with the storage containers has to be considered. Since atomic force microscopy is well suited for imaging topography of glass surfaces, any kind of alterations (e.g., corrosion or leaching) can be observed by this technique. Using XPS, the glass surface can be analyzed in terms of chemical reactions, such as ion exchange or the enrichment of ions on the surface. The analysis of the inner surface of vials with XPS and AFM enables the differentiation between various glass qualities. Topological effects of surface treatment, as well as the hydrolytic resistance of glass, are important factors that have to be taken into account when evaluating the compatibility between glass container surfaces and drugs. Enrichment of ions on the surface leads to a decreased hydrolytic resistance of glass and thus to an increased release or leaching of ions into solution, where they can react further with drug molecules.

Acknowledgments

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