Measuring the Surface Area of Aluminum Hydroxide Adjuvant

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ABSTRACT: The traditional method of determining surface area, nitrogen gas sorption, requires complete drying of the sample prior to analysis. This technique is not suitable for aluminum hydroxide adjuvant because it is composed of submicron, fibrous particles that agglomerate irreversibly upon complete removal of water. In this study, the surface area of a commercial aluminum hydroxide adjuvant was determined by a gravimetric/ FTIR method that measures the water adsorption capacity. This technique does not require complete drying of the adjuvant. Five replicate determinations gave a mean surface area of 514 m^2/g and a 95% confidence interval of 36 m^2/g for a commercial aluminum hydroxide adjuvant. The X-ray diffraction pattern and the Scherrer equation were used to calculate the dimensions of the primary crystallites. The average calculated dimensions were $4.5 \times 2.2 \times 10$ nm. Based on these dimensions, the mean calculated surface area of the commercial aluminum hydroxide adjuvant was 509 m^2/g, and the 95% confidential interval was 30 m^2/g. The close agreement between the two surface area values indicates that either method may be used to determine the surface area of aluminum hydroxide adjuvant. The high surface area, which was determined by two methods, is an important property of aluminum hydroxide adjuvants, and is the basis for the intrinsically high protein adsorption capacity. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:1702–1706, 2002
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INTRODUCTION

The surface area of aluminum hydroxide adjuvant affects the adsorption of antigens as well as the physical properties of vaccines. Unfortunately, reliable surface area values for aluminum hydroxide adjuvant are not available. This is due to the morphology of aluminum hydroxide adjuvant. Transmission electron photomicrographs[1,2] reveal that the primary particles are needle-like fibers having a diameter of approximately 2 nm. The primary particles form stable aggregates having diameters ranging from 1 to 5 μm. When a suspension of such particles is completely dried,

the primary and secondary particles agglomerate irreversibly.[3] Nitrogen BET, the standard method for measuring surface area, is based on the sorption of nitrogen gas. This method requires the removal of all of the water from the surface prior to exposure to the nitrogen gas. Completely drying aluminum hydroxide adjuvant results in an artificially low surface area.

The particle morphology of aluminum hydroxide adjuvant has limited the characterization of surface area to general statements. For example, the monographs[4] for commercial adjuvants such as Alhydrogel and Rehydragel HPA state that: "its high surface area gives it a high adsorptive capacity for antigens."

A technique that combines infrared spectroscopy with gravimetric analysis has been recently developed[5] to measure the surface area of clay minerals. Although swelling clays also

agglomerate irreversibly when all of the water is removed, the technique does not require complete drying of the sample. This technique will be applied to aluminum hydroxide adjuvant.

Mathematical analysis of the X-ray diffractogram of a crystalline material can yield the dimensions of the smallest repeating unit or primary crystallite. Because the primary particles of aluminum hydroxide adjuvant observed by transmission electron microscopy have a fibrous morphology and are very small, the surface area calculated from the X-ray diffraction pattern may represent the actual surface area of aluminum hydroxide adjuvant.

MATERIALS AND METHODS

Commercially available aluminum hydroxide adjuvant (Rehydragel HPA, Reheis, Berkeley Heights, NJ) was used.

X-ray Diffraction

The X-ray diffractogram of each adjuvant was obtained using a random powder mount. The sample was air dried at room temperature and examined from 5 to 70° 2θ using CuKα radiation at a scan rate of 1°/min (Siemens, Madison, WI). The width of the X-ray diffraction bands were used with the Scherrer equation[6] to calculate the dimensions of the primary crystallites.

Water Sorption

A schematic diagram of the gravimetric FTIR spectrometer is shown in Figure 1. The system consisted of a microbalance (D200, Thermo Cahn, Madison, WI), an infrared transmission cell connected to a flowing gas manifold and a FTIR spectrometer (Model 1600, Perkin Elmer, Norwalk, CT) in which the IR cell was placed. A detailed description of this technique and its application to water sorption measurements of clay minerals has been recently reported.[5] The IR cell consists of a 7.5-cm path-length gas cell fitted with two 50 mm (diameter) × 3 mm (thickness) ZnSe windows. A self-supporting film of adjuvant was suspended from the weighing arm of the microbalance. The self-supporting film of adjuvant was mounted in an aluminum foil holder to support the film and to keep it flat and perpendicular to the IR light path.
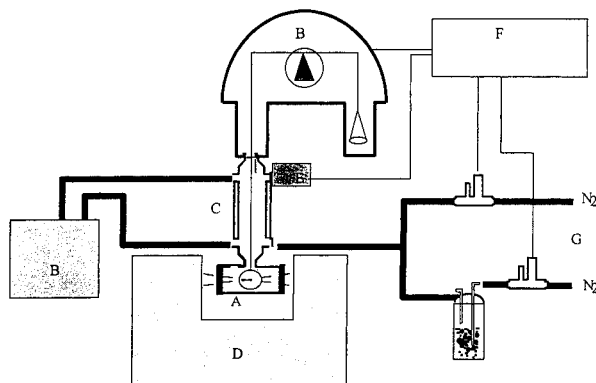

Figure 1. Schematic of the gravimetric FTIR apparatus showing the (A) 7.5-cm pathlength IR cell, (B) microbalance with sample suspended from weighing arm, (C) water bath, (D) FTIR spectrometer, (E) relative humidity and temperature probe, (F) data acquisition computer, (G) flowing gas manifold.

The relative humidity (RH) in the cell was controlled by a flowing-gas manifold using two controllers (Mass-Flo, MKS Instruments, Andover, MA) connected to a source of dry or wet nitrogen gas. The wet nitrogen was saturated with water vapor by flowing the nitrogen gas through a water bubbler at 40°C. The relative proportion of the dry or wet nitrogen was adjusted to maintain a specific relative humidity. The total flow rate was constant at 100 sccm (standard cm³ min⁻¹). The buoyancy effect and water sorption of the holder were measured using the empty holder.

The temperature of the vapor chamber was maintained at $22 \pm 1°C$ using a circulating water bath. The RH and temperature were monitored on-line by a humidity probe (HMP35a, Vaisala, Woburn, MA). The probe was calibrated using saturated solutions of lithium chloride (11.3% RH) and potassium chloride (85.1% RH) at $20 \pm 1°C$.

The water sorption experiment was started at 0% RH. The RH was increased in 5% increments in the 0–50% RH range and by 10% increments in the RH range from 50–100%. Samples usually reached equilibrium within 1 h after each RH step.

FTIR spectra were collected using a DTGS detector by coaddition of 32 individual scans in the range of 4400–450 cm⁻¹ with a spectral resolution of 4.0 cm⁻¹. The Grams Analyst program (Galactic Software, Thermo-Galactic, Salem, NH) was used to control the FTIR spectrometer. The program was synchronized with the Lab View program (National Instruments, Austin, TX) to

ensure that the spectra were taken 5 min before the end of each RH step. The corresponding RH and sample weight recorded in the sampling window were averaged.

RESULTS AND DISCUSSION

X-ray Diffraction

The X-ray diffraction (XRD) pattern of the aluminum hydroxide adjuvant is shown in Figure 2. The XRD pattern exhibits six broad reflections that correspond to poorly crystalline aluminum oxyhydroxide, which is known mineralogically as poorly crystalline boehmite.[1] The width of these reflections is related to the size of the primary crystallites with an increase in width corresponding to a smaller particle size The width of these reflections is related to the size of the primary crystallites by the Scherrer equation.[6] Based on the observed widths of the XRD reflections, the average dimensions of the primary crystallites were calculated by the Scherrer equation to be $4.5 \times 2.2 \times 10$ nm along the crystallographic axes a, b, and c, respectively. Based on these dimensions and the density of aluminum oxyhydroxide[7] (3.05 g cm^{-3}), the calculated surface area of the aluminum hydroxide adjuvant was 509 m^2/g and the 95% confidence interval was 30 m^2/g.

Water Sorption

The water sorption isotherm was obtained from a self-supporting film of aluminum hydroxide adjuvant using the gravimetric FTIR cell shown in Figure 1. The weight of the sample was recorded at each RH step (Fig. 3, left) and a corresponding
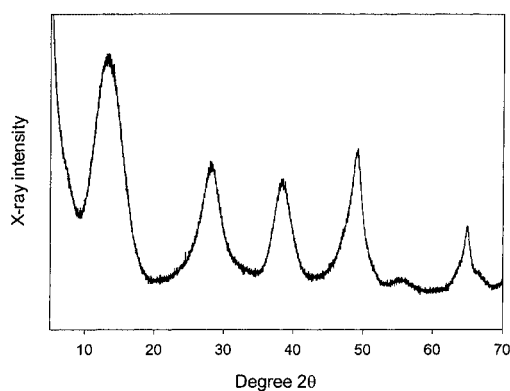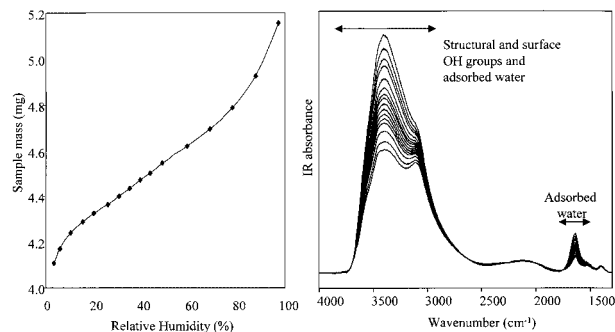
Figure 3. Effect of relative humidity on the weight of the aluminum hydroxide adjuvant (left) and the FTIR spectra of aluminum hydroxide adjuvant at RH values ranging from 0 (bottom) to 100% (top) (right).

FTIR spectrum was recorded (Fig. 3, right). The dominant features in the FTIR spectra are the broad, intense OH-stretching bands in the 3800–2600 cm^{-1} region and the H−O−H bending band in the 1700–1600 cm^{-1} region. The ν(OH) bands in the 3800 to 2600 cm^{-1} regions are comprised of spectral contributions from both sorbed water and structural OH groups of the aluminum hydroxide adjuvant. In contrast, the band at 1640 cm^{-1} is assigned to the H−O−H bending mode of sorbed water only. The hydroxyls of the adjuvant do not contribute any intensity to this band. Consequently, the 1640 cm^{-1} band can be used to quantitatively determine the amount of water sorbed to the sample.[5]

The combined approach of using gravimetric and FTIR analysis provides a unique benefit in the study of high surface area, hydrophilic materials such as aluminum hydroxide adjuvant, as the method does not require complete desiccation of the sample to determine the dry weight of the sample. In other words, the microbalance measures the total mass of the sample and the FTIR spectra can be used to quantitatively determine the total water content. This is illustrated in Figure 4, which shows the absorbance of the 1640 cm^{-1} band (HOH bending band) (Figure 3, right) as function of the sample weight. The dry weight of the sample was obtained by extrapolation. The sample retained a significant amount of moisture even at very low RH. In traditional water or nitrogen sorption experiments,[8] the sample must be heated and/or evacuated to determine the dry weight. There are two significant problems with the traditional approach. First, once the sample is dried, its structure has been irreversibly changed. Second, it is doubtful that
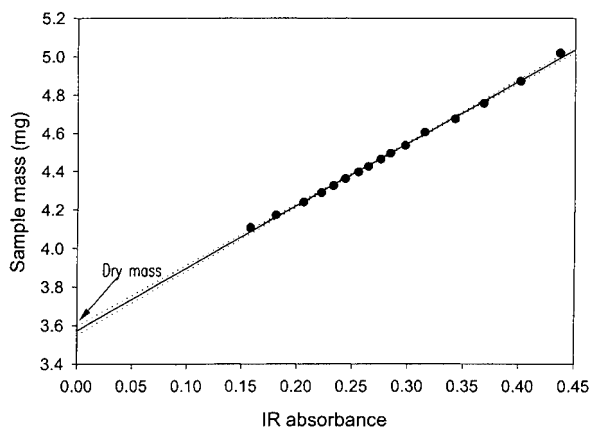
Figure 2. X-ray diffraction pattern of aluminum hydroxide adjuvant.

Figure 4. Weight of the aluminum hydroxide adjuvant versus the absorbance at 1635 cm^{-1}.

all of the water can be removed from the sample even after heating and evacuation.[9]

After the dry weight of the sample was obtained, a water sorption isotherm was plotted (Fig. 5) expressing the water content as mg H_2O/g adjuvant. Application of the BET equation[10] to the water adsorption isotherm yields a surface area of 514 m^2/g and a 95% confidence interval of 40 m^2/g for five replicates.

Comparison of Surface Area by X-ray Diffraction and Water Sorption

Application of X-ray diffraction and water sorption to a commercial aluminum hydroxide adjuvant yielded virtually identical surface area values of 510 m^2/g. To our knowledge, this is the first reported surface area of aluminum hydroxide adjuvant. The similar surface area obtained by each method validates the use of each technique.
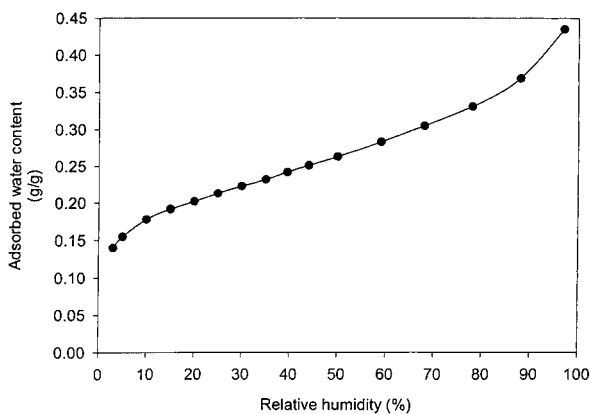

Figure 5. Water sorption isotherm of aluminum hydroxide adjuvant.

The results indicate that the dimensions of the primary crystallite determined by the XRD are similar to the dimensions of the actual primary particle. The water sorption data provide a measure of the total exposed surface area. The XRD-derived surface area value calculated using the dimensions of the primary crystallites agree with this value. Although the primary crystallite dimensions rarely coincide with the dimensions of the primary particles observed using electron microscopy, we believe that the agreement found in this study is related to the fibrous morphology and exceedingly small size of the nanosized particles.

The high surface area obtained by both methods is consistent with the high adsorption capacity of aluminum hydroxide adjuvant for various proteins: 1.6–3.1 mg bovine serum albumin/mg Al,[11–16] 2.6 mg ovalbumin/mg Al,[17] 1.9 mg α-lactalbumin/mg Al,[18] and 1.1 mg myoglobin/mg Al.[18]

A surface area of 510 m^2/g is unusual for a crystalline material and approaches the surface area values reported for expandable clay minerals that range from 600 to 800 m^2/g.[19]

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