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Adsorption of water on cyclosporin A, from zero to finite surface coverage

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Summary

A gas-chromatographic technique was used to measure the adsorption of water at zero and finite surface coverage of the orthorhombic form of cyclosporin A. For calculation of the surface area, the BET adsorption isotherms of water at 373 K and nitrogen at 77 K were utilized. When nitrogen was used as adsorbate a lower value for the specific surface area was obtained. This could be explained in terms of the size and shape of the adsorbate molecule and its ability to access the internal surface of the adsorbent. Results obtained with the scanning electron microscope revealed small apertures on the adsorbent surface.

Introduction

The interaction between water and pharmaceutical excipients or drug substances is of essential importance for the chemical, physical and microbiological stability of the product (Ahlneck and Zografi, 1990). Some characteristics known to be altered by the association of solids with water include rates of chemical degradation, crystal growth and dissolution, dispersibility and wetting, powder flow, lubricity, powder compactibility, and compact hardness.

Water can associate with solids in two ways. Water molecules can interact only with the surface of the solid (adsorption) or penetrate the bulk solid structure (absorption). When both adsorption and absorption occur, the term sorption is often used. To understand the effects water can have on the properties of solids requires an understanding of the location and mobility of molecules of water as well as its physical state. The difference in mobility can be observed through such measurements as heats of sorption, freezing point, NMR, dielectric properties, and diffusion.

The many different static techniques that have been developed for the determination of the quantity of gas adsorbed onto a solid surface can be divided into volumetric methods (measurement of the amount of vapor removed from the gas phase), and gravimetric methods (direct measurement of the increase in the mass of the adsorbent). In some cases, direct analysis of water content by methods such as Karl Fisher titration, X-ray crystallographic analysis, thermal

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analysis and inverse gas chromatography (IGC) may be advantageous.

IGC represents a dynamic technique that can be used to determine the amount of adsorbate on the adsorbent surface, provided that there is negligible competitive adsorption of the carrier gas. The word 'inverse' indicates that the component of interest is the stationary phase (adsorbent), rather than the injected volatile substances (adsorbate). The technique is well-established and is described in earlier papers (Laub and Pecsok, 1978; Conder and Young, 1979; Lloyd, 1989). Gas chromatography differs from static methods in the rapidity of measurements with simple standard apparatus which does not need to be evaluated: in addition, the accuracy is often retained or even considerably increased. The experiments can be conducted over a wide range of temperatures. It differs from the calorimetric method in adsorption measurements in that very low surface coverage and much higher temperatures may be used.

The aim of this work was to study the interaction between water and cyclosporin A (CyA) using IGC.

Materials and Methods

A laboratory-constructed gas chromatograph was employed for the IGC measurements. The precision, accuracy and potential sources of error of such a system have been discussed in detail elsewhere (Laub et al., 1978; Djordjevic et al., 1986). A water-bath (Julabo, Switzerland), with Julabo M liquid used as a heating fluid, was used for temperatures up to 393 K. The detector was a Gow-Mac (Ireland) thermal conductivity unit, and the injection system was a Varian injection port. The recorder was from Philips (model PM 8252A). Helium of 99.99% purity, obtained from Carbagas (Switzerland), was used as the carrier gas throughout the experiment. The reference and sample carrier flow rate was controlled with a Chrompak flow controller and pressure regulator. The column inlet pressure was read to 0.01 psi with a Chrompak digital pressure gauge. The carrier gas flow rate was measured to 0.05 cm^3 min^{-1} with a water-jacketed soap-bubble burette. Cyclosporin A was obtained from Sandoz Pharma (Switzerland).

Prior to data acquisition by IGC, the solid material (CyA, particle size 300–400 μm) was filled into an 80×0.3 cm i.d. glass column and dried for 24 h at 393 K in a stream of He, at a flow rate of 3 cm^3 min^{-1}. For zero surface coverage experiments, the lowest detectable quantity of adsorbate vapor was injected with a Hamilton 10 μl syringe, an average of at least three runs being taken. The raw retention times were measured with a stopwatch. Peak areas were determined by cutting and weighing.

Surface area measurement, by the BET method with nitrogen as adsorbate at a temperature of 77 K, was performed using the commercial instrument Quantasorb Jr. (Quantachrom Corp.). Karl Fisher apparatus was purchased from Metrohm Hervisan (Switzerland). Thermal analysis was performed using a TGA-7 thermogravimetric analyzer (Perkin-Elmer). An X-ray powder diffractometer (model XPS2000), from Scientac Inc. (Santa Clara) was used. The scanning electron microscope used in this study was a Stereoscan 180S (Cambridge). Samples were gold sputter-coated prior to examination to render them electrically conductive and images were obtained using secondary electron detection.

Adsorbate retention at zero surface coverage

Adsorbate net retention volumes were computed from the expression

$$V_n = (t_r - t_a) j F_c \tag{1}$$

where j is the James-Martin carrier compressibility correction factor, F_c represents the carrier corrected flow rate, and t_a is the retention time of a non-retained compound (e.g., air). Surface partition coefficients, K_s, were then calculated from the relation

$$K_s = V_n / A_s \tag{2}$$

where A_s is the adsorbent surface area (m^2 g^{-1}). From the temperature dependence, T, of the partition coefficient, thermodynamic data describing the retention (adsorption) process were derived. The differential heat of adsorption of the adsorbate at zero surface coverage, (i.e., the standard-state enthalpy of adsorption $-\Delta H_A^\circ$), was obtained form the relation

$$d(\ln K_s)/d(1/T) = -\Delta H_A^\circ/R \quad (3)$$

provided that ΔH_A° is temperature independent. The standard free energy change ΔG_A° for the isothermal adsorption of 1 mol of adsorbate is related to the partition coefficient

$$\Delta G_A^\circ = -RT \ln(Kp_{s,g}/P_s) \quad (4)$$

where R is the gas constant, $p_{s,g}$ denotes a reference pressure (101.3 kN m^{-2}), while P_s is a reference two-dimensional surface pressure whose standard state is arbitrary. The usual value for P_s is 0.338 mN m^{-1}, proposed by De Boer (1953), which arbitrarily defines the standard surface pressure at which the average distance of separation between molecules in the adsorbed state equals that in the standard gas state. The standard state entropy of adsorption ΔS_A° is calculated from the relation

$$\Delta S_A^\circ = (\Delta H_A^\circ - \Delta G_A^\circ)/T \quad (5)$$

Adsorbate retention at finite surface coverage

The amount of adsorbed material q corresponding to a particular partial pressure p is given by (Kiselev and Yashin, 1969):

$$q = (nS_a)/(wS_p) \quad (6)$$

where n is a known amount (mol) of adsorbate injected, S_a represents the chart area bounded by the diffuse profile of the chromatogram air peak (used as non-retained compound) and the peak height h, while S_p is the chart area under the peak. The partial pressure of the adsorbate in the gas phase is recovered via the relation

$$p = (nshRT)/S_pF_c \quad (7)$$

where s is the recorder chart speed.

Results and Discussion

Finite surface coverage

Cyclosporin A is a cyclic undecapeptide, having extremely low solubility in aqueous media and high solubility in non-polar organic solvents. The synthesis of CyA has been described in detail elsewhere (Wenger, 1984). The X-ray diffrac-

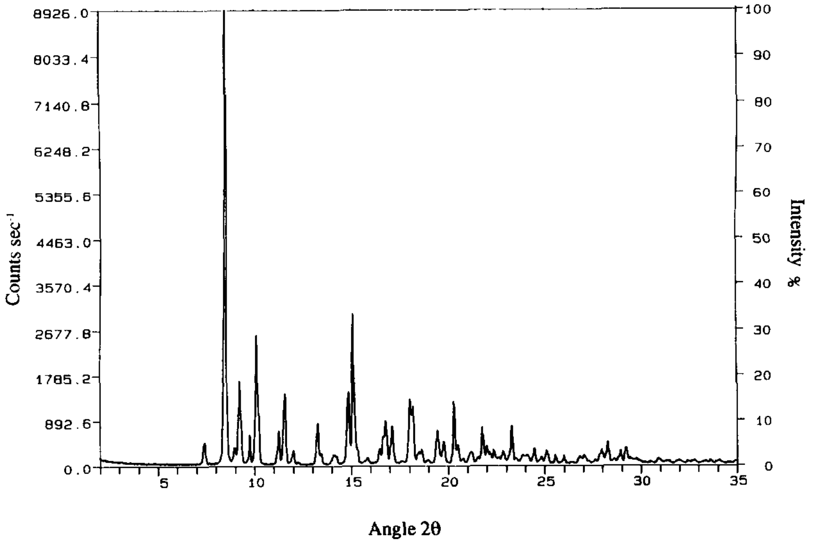

Fig. 1. X-ray powder diffractogram for cyclosporin A.

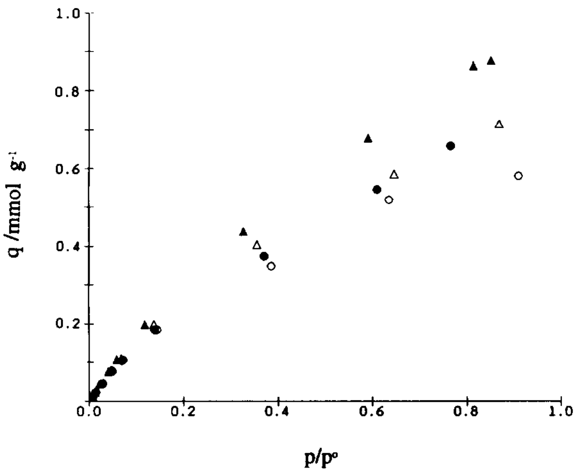

Fig. 2. Adsorption isotherms for water on cyclosporin A at (▲) 363 K, (△) 373 K, (●) 383 K and (○) 393 K.

togram of its orthorhombic form, crystallized from polyethylene glycol 300, and used in this study is depicted in Fig. 1. A detailed crystal-structure analysis of CyA has been discussed previously (Loosli et al., 1985).

In measuring sorption-desorption isotherms, by IGC, using the elution by characteristic point technique, detailed elsewhere (Conder and Young, 1979), it is important to ensure that the effect of non-ideality is small in comparison with non-linearity (Kiselev and Yashin, 1969). This condition was fulfilled by ensuring that the self-sharpening side of the peak was vertical and that the diffuse rear profiles of the peaks resulting from the injection of different quantities fell on a common curve. Adsorption isotherms derived from chromatographic data are presented in Fig. 2. The isotherm belonged to Type I according to Brunauer's classification (Brunauer, 1945). This type of isotherm is characteristic of microporous adsorbents, having relatively small external surfaces, the limiting uptake being governed by the accessible micropore volume.

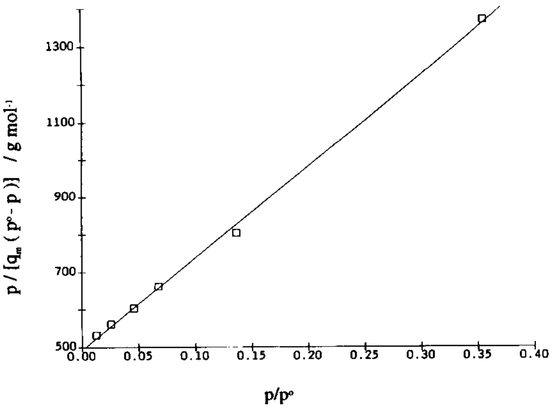

Fig. 3. BET transform of the water isotherm at 373 K.

The surface area of the CyA adsorbent was determined from the calculated monolayer capacity, q_m. This quantity was computed from the adsorption data at 373 K using the BET isotherm (Brunauer et al., 1938) which is given by equation

$$p/[q(p^\circ - p)] = (1/q_m C) + (C-1)p/q_m C p^\circ \tag{8}$$

where p and p° represent the pressure of water in the column and the vapor pressure of the water at the column temperature, respectively, and C is an empirical parameter which is related to the heat of adsorption and the shape of the isotherm in the region of monolayer formation. A plot of $p/[q(p^\circ - p)]$ vs p/p° yielded straight line up to a relative pressure of 0.36 (Fig. 3). From the slope and intercept the monolayer capacity, q_m, equal to 3.45×10^{-4} mol g^{-1} and BET constant C equal to 5.9 were determined. The specific surface area of the adsorbent, A_s, was calculated using the following equation,

$$A_s = a_m N q_m \tag{9}$$

where a_m denotes the area occupied by each molecule and N is Avogadro's constant. It is difficult to estimate the area which an adsorbed molecule occupies on a solid surface. In this study the adsorption areas of a water and a nitrogen molecule were taken as 11.4×10^{-20} and 16.2×10^{-20} m^2, respectively (Brunauer et al., 1958; McClellan and Harnsberger, 1967). The value for the adsorbent surface area, obtained from the water isotherm (23.72 m^2 g^{-1}), was two orders of magnitude higher than that determined from the nitrogen isotherm (0.30 m^2 g^{-1}). The internal surface determined by gas adsorption may depend on a dimension of the adsorptive molecule, e.g. its size and shape.

If the pores contain constrictions narrow enough to hinder the free passage of molecules through them, the measured uptake may then correspond to adsorption on only a fraction of the total available surface. The diffusivity, hence the kinetic selectivity and, in extreme cases, the molecular sieve properties are determined mainly by the free diameters of the windows in the intracrystalline channel structure. Due to the effects of vibration of both the diffusing molecule and the crystal lattice, these windows may be penetrated by molecules with diameters which are somewhat greater than the normal aperture. If a solid contains micropores, capillaries which have a width not exceeding a few molecular diameters, the adsorptive behavior will show considerable inconsistency when compared with that of non-porous or macroporous solids. In particular, the potential fields from opposite walls will overlap so that the attractive force acting on adsorbate molecules will be increased as compared with that on an open surface. The adsorption isotherm will accordingly be distorted in the direction of an increased adsorption.

Variations in the surface area obtained with two different adsorbates are due to the sieving effect. The value determined with nitrogen as the adsorbate could be regarded as an external surface area, the pore diameter, on average, being too small for the penetration of nitrogen molecules. The obtained surface area with water as adsorbate should represent total approachable area, external and internal, the latter being predominant. A water molecule has access to the internal surface area of the adsorbent, the cross-sectional area of a water molecule being smaller than that of a nitrogen molecule. The same discrepancy in specific surface area was reported for microcrystalline cellulose when nitrogen and water were used as adsorbates (Nakai et al., 1977). Molecular sieve effects cannot be entirely excluded even if the average width of the pores is several molecular diameters. Almost inevitably, there will be a distribution of pore sizes and the proportion of the pores open to a given molecule will be smaller the larger its molecular size. Furthermore, this supports the theory that the basic geometrical parameter marking a microporous adsorbent is the volume of the micropores rather than their external surface (Dubinin, 1967).

It should be pointed out, that in our work, the values obtained by BET analysis from nitrogen adsorption were measured at 77 K, while IGC was performed at 373 K. This temperature difference may result in a change in pore diameter. If the pores of a microporous solid contain constrictions which are only slightly wider than the minimum dimension of the adsorbate molecules, as previously discussed, the passage of the molecules through the constrictions will be hindered. In reaction-kinetic terms, the extent of passing through the constrictions in a given time, to enter the space beyond, will increase rapidly with rise in temperature, so that the measured adsorption will correspondingly increase as temperature increases. In other words, the passage through the constriction will be ‘activated’ (Djordjevic and Laub, 1988). Each molecule, in the gas phase, has to surmount an energy barrier in order to gain entry into the cavity.

A scanning electron microscope was used to obtain more information about the topography of the investigated adsorbent. The electron micrograph (Fig. 4) unveils small openings on the crystal surface. If it is assumed that the availability of surface adsorption sites (their existence and/or their ease of access) was a major factor controlling wettability, then ‘droplet’ formation would be more favorable than monolayer formation (Buckton and Beezer, 1988). These droplets formed on the surface served as a binding medium and thus caused particles to agglomerate. This was observed through high column inlet pressure at the start of the column conditioning. After conditioning for 24 h, the inlet pressure dropped significantly and remained constant, implying that the adsorbent was cleared from adsorbed water.

The amount of water present in an adsorbent prior to its use for IGC, determined by Karl Fisher titration and by thermogravimetry (TGA), was 1.4 and 0.67 wt%, respectively. Incomplete removal of sorbed and occluded water when the TGA technique was used may be responsible for this discrepancy.

Fig. 4. Scanning electron micrograph of cyclosporin A. Magnification, ×5000; scale bar, 10 μm.

Since most adsorbents have non-uniform surfaces, adsorption at low coverage takes place on the energetically more active sites. From a plot of the differential heat of adsorption against fraction of the surface covered, a diminishing function due to the decrease in the number of high-energy adsorption sites is foreseen. At higher surface coverage, lateral interaction on the surface must also be considered. The more adsorbate is present on the surface, the greater is the interaction energy which results. These two effects are in opposite directions; if and when they balance each other, isosteric heat of adsorption is constant.

Application of the Clausius-Clapeyron equation to the adsorption isotherms at different temperatures yielded the isosteric, i.e., constant loading, heats of adsorption

$$q_{st} = -R[d(\ln p)/d(1/T)]_x \qquad (10)$$

Fig. 5 depicts calculated values for q_{st} (open circles), determined graphically from plots of ln p vs $1/T$, together with data for the heat of adsorption at zero surface coverage (filled circle) and heat of liquefaction of H_2O (horizontal dashed line). The heat of adsorption at zero surface coverage represents solely the adsorbate-adsorbent interaction and will be discussed later. The higher value for the heat of adsorption at

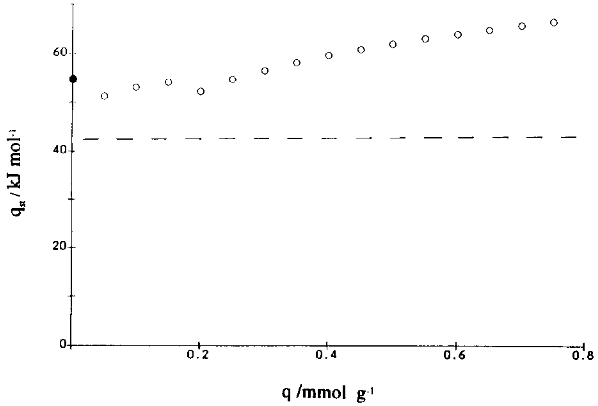

Fig. 5. Isosteric heat of adsorption of water as a function of the cyclosporin A surface coverage at 373 K. Filled circle, ΔH_A from measurements at zero surface coverage; horizontal dashed line, molar heat of liquefaction ΔH_L.

zero surface coverage and the fairly constant isosteric heat of adsorption, above the heat of liquefaction of water (dashed line in Fig. 5), lead to the conclusion that a meaningful number of energetically high sites are available for adsorption. A characteristic feature of adsorption on microporous adsorbents is a significant rise in the adsorption energy as compared to the corresponding values for large porous or nonporous adsorbents of a similar chemical structure (Dubinin, 1967).

The most important effect of the adsorption of a vapor upon a solid is the reduction of free surface energy, Π, which accompanies the adsorption. This quantity Π, the spreading pressure, is by definition

$$\Pi = \gamma_s - \gamma_{sv} \tag{11}$$

where γ_s and γ_{sv} are the surface free energy of the solid at the solid-vacuum interface and the solid-vapor interface, respectively. Bangham (1937) and Bangham and Razouk (1937, 1938) were the first to show that the decrease of the free surface energy could be evaluated by the use of the Gibbs adsorption equation. The integration of this equation is expressed by the relation

$$\Pi = \gamma_s - \gamma_{sv} = RT\int_0^p Q \, d(\ln p) \tag{12}$$

where R denotes the gas constant, T is the absolute temperature and Q represents surface concentration. To solve the integral, the adsorption isotherm at 363 K was first fitted to a polynomial of the form (Katz and Gray, 1981):

$$Q = \sum_{j=1}^{j} k_j p^j \tag{13}$$

using a regression technique. The k's in Eqn 13 represent the coefficients of the six-term polynomial, and the isotherm is assumed to pass through the origin. Combining Eqns 12 and 13 yields the following expression

$$\Pi = RT \sum_{j=1}^{j} (k_j/j) p^j \tag{14}$$

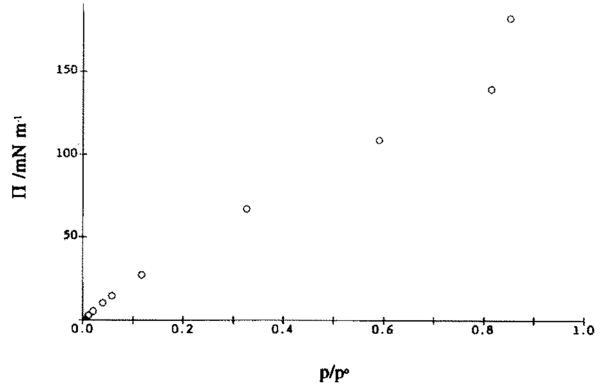

Fig. 6. Spreading pressure of water on cyclosporin A as a function of relative partial pressure at 363 K.

It is important to consider the way in which the lowering of the free surface energy of a solid alters with the relative pressure of the vapor. On plotting Π vs p/p_0 (Fig. 6), the curve was found to exhibit two points of inflection. The non-porous solids have only one point of inflection. The flattening of the curve for the porous solid at the highest pressures is due to the fact that the pores of the solid are filled with the adsorbate, so reducing the available adsorbing area. Thus, values for the reduction in free energy per unit area become questionable when applied to porous materials at high relative pressures, i.e., in this case at values of p/p_0 of around 0.8.

Zero surface coverage

At low surface coverage, any effects of interaction between neighbouring adsorbed molecules can be neglected. The forces involved in physical adsorption include both Van der Waals forces (dispersion-repulsion) and electrostatic interactions (polarization, dipole, and quadrupole interaction). The Van der Waals contribution is always present while the electrostatic contributions are significant only in the case of adsorbents which have an ionic structure, such as zeolites. For the sorption of small dipolar molecules, such as H_2O, on this type of adsorbent, the electrostatic contribution may be very large, leading to unusually high heats of adsorption. Furthermore, the adsorption is quite specific and the rate is often controlled by an activated diffusion process, giving the appearance of a slowly activated chemisorption, even though the actual surface adsorption may be rapid. The constraint of pore opening acts as an energetic barrier which must be surmounted by the 'activation' energy of the adsorbate molecule so that it can penetrate into the pore.

The water partition coefficient, K_s (ml m^{-2}), in the temperature range from 363 to 393 K (in increments of 10 K) was 0.953, 1.412, 2.219 and 3.830, respectively. The differential heat of adsorption, $\Delta H_A^o = -54.944$ kJ mol^{-1}, was calculated from the slope of ln K_s vs reciprocal temperature. It was higher than the heat of liquefaction, $\Delta H_L = -41.20$ kJ mol^{-1}, indicating the presence of high-energy sites. If the transannular hydrogen bonds in the CyA molecule (Loosli et al., 1985) are broken at the elevated temperature, then intermolecular hydrogen bonding should control retention of the adsorbate (water) molecule. The adsorbent-adsorbate hydrogen bond interaction is usually distinguished by non-symmetrical peaks (strong tailing). In our experiment, throughout a linear portion of the adsorption isotherm, the water peak was symmetrical with a skew ratio of 0.95 (ratio of slope of peak trailing edge to that of leading edge at point of inflection). Furthermore, in refining a crystal structure of CyA, Loosli et al. (1985) discovered two water molecules which just filled 'empty space', without engaging in hydrogen bond or other specific interactions. This supports the hypothesis of a sieving effect as the major mechanism of retention of water on the CyA adsorbent.

The standard free energy of adsorption at 373 K, $-\Delta G_A^o = 20.15$ J mol^{-1} K^{-1}, was calculated using Eqn 4. Additional information about the surface of a solid can be obtained by considering the standard entropy of adsorption ΔS_A^o. Upon adsorption from the gas phase, one translational degree of freedom is lost in favor of a vibrational mode to the plane of the surface, while free rotation in the gas phase is reduced to hindered rotation in the adsorbed state. As the entropy in the adsorbed state is higher than that in the solid or liquid state, adsorbed molecules therefore have a far greater degree of freedom than molecules of a liquid or solid. Subsequently, the absolute entropies of adsorption of the adsorbates were calculated according to the method of De Boer (1953). The three-dimensional translational entropy of the adsorbate as an ideal gas is given by

$$^3S_t = R\ \ln(M^{3/2}T^{5/2}) - 9.62 \qquad (15)$$

where M is the molar mass of the adsorbate and the entropy, ^3S_t, is expressed in J mol^{-1} K^{-1}. Similarly, considering the adsorbate to be an ideal gas having unrestricted freedom of movement in two directions and no freedom of movement in a direction perpendicular to the surface of the adsorbent, the two-dimensional translational entropy is calculated from the equation

$$^2S_t = R\ \ln(MTA) + 275.32 \qquad (16)$$

where A is the area of the adsorbent available for adsorption of an adsorbate molecule in its standard state; defined by De Boer (1953) as $A = 4.08 \times T \times 10^{-16}$ cm^2. The calculated values for ^3S_t, ^2S_t together with $S_t = (^3S_t - {}^2S_t)$ were 149.0, 103.2 and 45.8 J mol^{-1} K^{-1}, respectively. The change in standard entropy of adsorption $\Delta S_A^o = -93.2$ J mol^{-1} K^{-1} was greater than would be expected from the loss of a single degree of translational freedom S_t. This is undoubtedly due to the additional constraints of restricted translational, rotational and possibly vibrational freedom imposed on the adsorbate as a result of the geometrical constrictions in the micropores.

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