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Monte Carlo Studies of Drug Nucleation 1: Formation of Crystalline Clusters of Bicalutamide in Water

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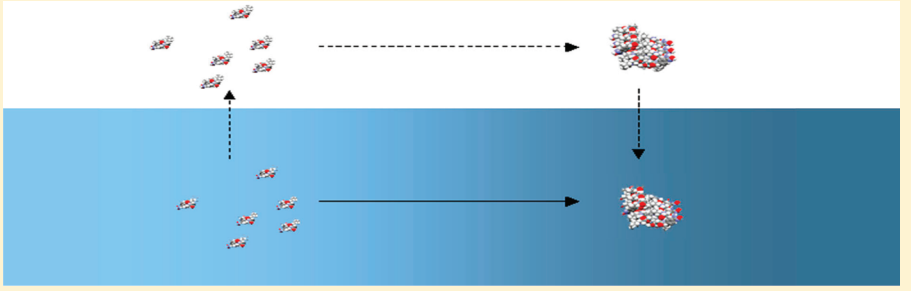
ABSTRACT:



A computational method of predicting the effects of the metastability of drug solutions is sought. A simple extension of our in silicio approach to thermodynamic drug solubility is tested on the drug bicalutamide for which we performed vapor pressure measurements complementing earlier measurements of aqueous solubility and crystal−water interfacial tension. The free energy of formation of an *N*-cluster of the drug molecule is estimated semiempirically by use of an Einstein model of the crystal in which experiment supplies the crystal structure, enthalpy of sublimation, and Einstein frequency of vibration. The rigid drug clusters with *N* from 2 to 14 are extracted from the bulk crystal by minimization of either cluster energy or radius of gyration. The free energy of hydration is estimated by Monte Carlo simulation combined with simplified response theory based on the OPLS-AA/COMPASS force field for the drug−water interaction and the TIP4P water model. The results have been interpreted in terms of an apparent crystal−water interfacial tension according to classical nucleation theory. The energy-minimal and radius of gyration-minimal clusters seem to give very similar crystal−water interfacial tensions for both the monoclinic and the triclinic polymorph. The interfacial tension of the monoclinic polymorph is significantly higher (by around 20%) than that of the triclinic polymorph in accordance with experiment. For the triclinic polymorph a substantial overestimation of the interfacial tension compared to estimates from crystal nucleation experiments is found, mitigated somewhat by an empirical scaling of the simulated binding energies and free energies of hydration.

1. INTRODUCTION

Crystallization is not only a very important industrial process but also an enticing area of research in its own right. For instance, crystallization is the major hurdle in structure determination of proteins needed to study the relation between their structure and function, and in industry crystallization is the major purification step, e.g., in the production of pharmaceuticals. Within the pharmaceutical industry there are problems of drug compounds being either extremely difficult to crystallize or difficult to crystallize selectively, that is they crystallize in an almost uncontrollable variety of forms depending upon the conditions. Many pharmaceutical substances reaching the market today are poorly soluble in water. Products of such compounds hence require more advanced formulatins to obtain a relevant exposure and therapeutic effect after, e.g., oral administration. In many of these formulations one utilizes a metastable state of the drug. Most of the metastable formulations are rather stable in the dry state for kinetic reasons. However, when exposed to water, either during their preparation or after administration, such drugs may rapidly convert to the most stable crystalline phase. Thus, to maintain the drug in the supersaturated state, understanding drug crystal nucleation is crucial.

Because the approach to equilibrium is often slow, substantial supersaturations can be achieved in the aqueous medium, in an apparent equilibrium between the amorphous phase and the solution. For this reason, we conducted research to allow this quantity, the amorphous solubility, of modern drug molecules to be computationally predicted on the basis of explicit Monte Carlo simulation of an amorphous phase and separately of a dilute aqueous solution of the molecule. Our "in silicio" approach[1,2] has been based on both brute force free energy perturbation simulations of molecular models and a simplified response (SR)

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theory allowing entropic effects to be included in the standard simulations used to determine interaction energies. Given the use of a simple empirical correction for the systematic part of the error of interaction energies in these complex systems, we were able to obtain the solubilities of an ensemble of more than 40 drug molecules with a root-mean-square deviation of about 1 order of magnitude. Thus, it was established that the amorphous solubility, a nonequilibrium property, can be estimated using equilibrium thermodynamical methods, i.e., regular equilibrium MC simulations. This is sufficient to provide a very important guidance in the development of new drugs.

Nevertheless, this guidance would be vastly improved if also the rate of relaxation toward true equilibrium could be ascertained, especially the rate of nucleation. While experimental measurement of the nucleation rate has been achieved for proteins, pharmaceutical agents, and inorganic compounds using either the induction time until detection of crystals[4−7] or the total number of crystallites detected after a fixed time[8] these methods are too time consuming to be feasible for routine application in industrial pharmaceutical development, where high throughput is of the essence. Some aid in these matters is provided by the conceptually simplest theory of nucleation, the classical nucleation theory (CNT) of Becker and Döring[9] and Zeldovich.[10] Central to this theory is the free energy of forming a cluster of molecules, but to determine this property, empirical input is required under the guise of the interfacial tension between the solution and the solid phases.

This leaves a great window of opportunity open for computer simulations to explore. However, since molecular simulations of the nucleation phenomenon are computationally too demanding for pharmaceutical crystals in contact with solvent for years if not decades to come, alternative strategies have to be developed. Deij et al. introduced one such method.[11] They constrained the conformational space of all molecules to the crystal lattice and neglected all interactions beyond the immediate crystal neighbors. Under these conditions they simulated the growth and decay of clusters in a grand-canonical Monte Carlo algorithm. From the statistics of the success of the growth and decay events observed, the size and free energy could be found within their model.

Duff and Peters[12] developed another interesting method to obtain the interfacial tension by cleaving clusters into halves. In their method, the work of cleaving a cluster into two halves and bringing them beyond interaction distance yields the surface tension, on the assumption that this quantity is invariant with cluster size. In their paper, this method is applied to a comparatively simple model Hamiltonian. For our more complex systems a single cluster of sufficient size to yield what may be a bulk limit interfacial tension is difficult enough to strain our computational resources. Resolving the properties and interactions of two such clusters from the single cluster to the noninteracting limit in water is likely to be beyond our capabilities.

With this report, we are starting a new program of research to explore the possibility of estimating the barrier for nucleation of poorly soluble drugs in water by an extension of the semiempirical MC simulation method developed for prediction of amorphous solubility. Bicalutamide, the chemical structure of which is shown in Figure 1, is an antiandrogen prescribed for the treatment of prostate cancer. It is also a good candidate for the semiempirical method outlined in this paper because of its chemical complexity, typical of modern drugs, and the availability of experimental data on its crystal structure and solubility.

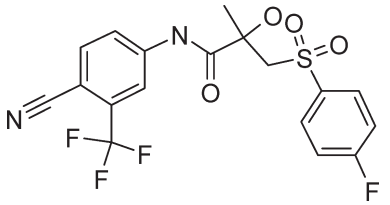

Figure 1. Chemical structure of bicalutamide.

Furthermore, its racemic structure crystallizes into two known polymorphs, the least stable of which, the triclinic polymorph, crystallizes preferentially out of aqueous solution. The stable polymorph crystallizes in the monoclinic crystal system. This therefore makes for a good comparison between the different apparent interfacial tensions between solution and the two polymorphs. We shall complement the experimental data on bicalutamide with our own measurements of its vapor pressure which is closely related to its solubility.

This paper is organized as follows. In section 2 we give a brief overview of the experimental methods and results. In section 3 the computational methodology is described in detail and results are presented and discussed concurrently. Finally, we draw our conclusions from this work in section 4.

2. EXPERIMENTAL MEASUREMENT

2.1. Materials and Methods. *2.1.1. Materials.* The monoclinic polymorph of bicalutamide (CSD code JAYCES01, CAS no. 90357-06-5) was obtained from AstraZeneca at 99.7% purity and used without further purification.

2.1.2. Sublimation Experiments. Sublimation experiments were carried out by the transpiration method detailed elsewhere.[13] In short, a stream of inert gas passes at known constant temperature and flow rate over the sample, slowly enough to ensure saturation of the carrier gas with the vapor of the sample. The vapor is allowed to condense downstream. The purity and mass of the condensate are then ascertained, allowing for calculation of the vapor pressure as proportional to the rate of mass accumulation. The equipment was calibrated using benzoic acid. The standard value of the obtained sublimation enthalpy (90.5 ± 0.3 kJ/mol) was in very good agreement with the value[14] recommended by IUPAC (89.7 ± 0.5 kJ/mol). Not less than a triple determination of the saturated vapor pressure was made at each temperature with the statistical error below 5%.

2.1.3. DSC Experiments. DSC experiments were carried out employing a DSC 204 F1 Phoenix differential scanning heat flux calorimeter (NETZSCH, Germany) with a high-sensitivity μ-sensor to determine the enthalpy and entropy of fusion. The sample was heated at a rate of 10 K min^{-1} in an argon atmosphere and cooled with gaseous nitrogen. The samples were placed into standard aluminum crucibles with lids. Temperature calibration of the DSC was performed against six high-purity substances, cyclohexane (99.96%), mercury (99.99+%), biphenyl (99.5%), indium (99.99%), tin (99.999%), and bismuth (99.9995%).

2.2. Results. The experimental vapor pressure p^a_{vap} of the monoclinic polymorph was regressed onto eq 1

$$\ln\left(\frac{p^a_{vap}}{Pa}\right) = A - \frac{B}{T} \qquad (1)$$

where $A = 33.1 \pm 0.2$ and $B = (14.1 \pm 7) \times 10^3$ K are constants, T is the temperature, and the functional form is taken from the

Table 1. Temperature Dependence of Saturation Vapor Pressure, Sublimation Thermodynamic Characteristics, and Thermophysical Parameters of Fusion Processes of Bicalutamide (monoclinic form)

T [°C]	$p_{vap}{}^{m} \times 10^2$ [Pa]	T [°C]	$p_{vap}{}^{m} \times 10^2$ [Pa]
103.0	1.36	129.0	15.1
107.0	2.07	131.0	18.1
110.0	2.60	133.0	20.6
115.0	4.16	136.0	27.5
116.0	4.46	139.0	35.0
119.0	6.08	142.0	45.4
121.0	7.58	145.0	57.7
125.0	10.5	148.0	74.1
127.0	12.9		
$\ln(p^m_{vap}[\text{Pa}]) = (33.1 \pm 0.2) - (14\,088 \pm 676)/T\sigma = 2.3 \times 10^{-2}$; $r = 0.9998$; $F = 43493$; $n = 17$			
T_m [K]	465.5 ± 0.2		
ΔH_{sub} [kJ·mol^{-1}]	117.1± 0.6		
ΔH_{fus} [kJ·mol^{-1}]	49.5 ± 0.5		
ΔS_{fus} [J·mol^{-1}·K^{-1}][a]	106 ± 1		
$\Delta G^{exp,298}_{hyd}$ [kJ·mol^{-1}][b]	−41.8		
p^m_{vap} [Pa][c]	7.15×10^{-7}	p^t_{vap} [Pa][d]	1.73×10^{-6}

[a] $\Delta S^T_{fus} = \Delta H^T_{fus}/T_m$. [b] $\Delta G^{exp,298}_{hyd}$ is the hydration Gibbs energy estimated from eq 4 (see text). [c] p^m_{vap} is the saturated vapor pressure at 298 K for the monoclinic polymorph obtained from the correlation equation. [d] p^t_{vap} is the saturated vapor pressure at 298 K for the triclinic polymorph obtained from eq 3 (see text).

Clausius−Clapeyron equation.[15] The experimental sublimation enthalpy at the temperature of measurement of the monoclinic form was ascertained directly from the B parameter as indicated in eq 2

$$\Delta H_{sub}(\text{monoclinic}) = N_A k_B B \tag{2}$$

where $N_A k_B$ is the gas constant (because we reserve R for "radius" later in the paper, we eschew the traditional denomination here). The thermodynamic characteristics of sublimation and fusion processes of the monoclinic form are shown in Table 1. The vapor pressure of the triclinic polymorphic form at 25 °C was estimated through the relation of eq 3

$$\frac{p^b_{vap}}{p^a_{vap}} \approx \frac{S^b_0}{S^a_0} \tag{3}$$

in which S^a_0 and S^b_0 denote the solubilities, known from experiment,[8,16] of the monoclinic and triclinic polymorphs, respectively, and p_{vap} the corresponding vapor pressure. Equation 3 is easily derived on the assumptions that the drug molecules do not interact with each other neither in the gas phase nor in the aqueous phase. These assumptions imply a low solubility for the drug molecule, which is very true of bicalutamide for which the solubility is in the lower micromolar range.[8,16] From the equilibrium relation in eqs 4 and 2

$$\frac{S^a_0 k_B T}{p^a_{vap}} = \exp\left(-\frac{\Delta G^{exp}_{hyd}}{k_B T}\right) \tag{4}$$

the experimental free energy of hydration of the monomer, ΔG^{exp}_{hyd}, was obtained. All these experimental quantities are summarized in Table 1. The free energy of hydration and the vapor pressure are the only two parameters that enter into our theoretical calculations.

3. CLASSICAL THEORY OF NUCLEATION

Let us first set up the basic assumptions and equations needed for our purpose. An implicit assumption in this work is that the crystallization of bicalutamide proceeds in a stepwise fashion, i.e., that all density fluctuations leading to recognizable clusters proceed through the attachment and detachment of single molecules only. Let us now denote a molecule as part of it if it is within a radius R of the center of mass of the constellation of molecules of said cluster. At this point we do not concern ourselves with the precise definition of R. The diffusive mass transport up to this distance, under the assumption that a steady state is at all times in effect, is the flow[8]

$$Q = 4\pi RD(C_b - S_0) \tag{5}$$

where D is the monomer diffusion coefficient, S_0 the solubility, in this case, the assumed concentration at the limiting distance, and C_b the bulk concentration. The flow in eq 5 can be straightforwardly partitioned into two terms; thus, $Q = Q_{in} - Q_{out}$, where the first term denotes the gross flow into and the second term the gross flow out of the cluster's sphere of influence. Because we must have $Q_{in} = 0$ when we do not have any bulk concentration present, we see that we have $Q_{in} = 4\pi RDC_b$ from eq 5.

The flow of monomers into clusters will establish a cluster distribution over all sizes and shapes of possible clusters. At thermal equilibrium, it is clear that such a distribution will be the Boltzmann distribution in the free energy of formation for such clusters. Away from equilibrium, this assumption becomes less tenable, but if the approach to equilibrium is slow, as is the case presently under investigation, a metastable equilibrium distribution very close to the true Boltzmann distribution may be assumed in effect for clusters smaller than a critical cluster representing a peak in the excess free energy of cluster formation. We may then write, if we take the reference state to be the solution of pure monomers and make no distinction between different shapes of the clusters, the cluster distribution function as the one-dimensional function $C_N = C_1 \exp(-\Delta G_N/k_B T)$. Because of our choice of reference

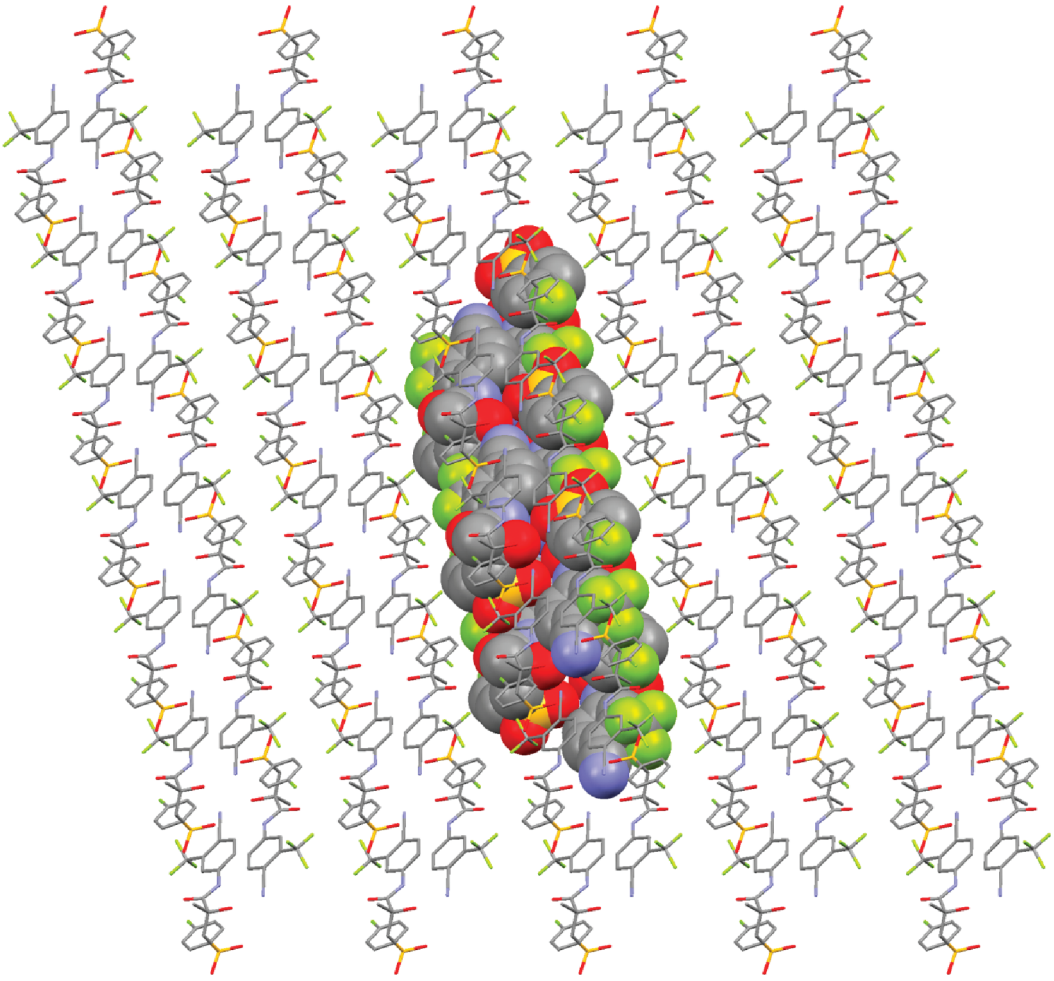

Figure 2. Illustration of the selection of the energy-minimal decamer from the bulk crystal structure. The cross section is of the (010) face of the triclinic polymorph JAYCES02.

state, it is clear that $\Delta G_1 = 0$. Here C_1 is a function whose dimensions are that of concentration and which usually is taken to denote the concentration of free monomers in the system, C_b. ΔG_N has a maximum as a function of N, which is the least likely cluster size, the so-called critical cluster, and this is the bottleneck of the crystallization process. Denoting by a superscript asterisk all properties pertaining to this critical cluster, we may write

$$J = \psi^* Q_{in}^* C_{N^*} Z \tag{6}$$

for the rate of formation of supercritical clusters, i.e., the nucleation rate. Here Z is the Zeldovich factor,[10] given by

$$Z = \sqrt{-\frac{1}{2\pi k_B T}\frac{\partial^2 \Delta G^*}{\partial N^2}} \tag{7}$$

which corrects approximately for the deviation of the Boltzmann distribution from the steady-state distribution under the assumption of monomer attachment and detachment. The parameter ψ^* is related to the probability that a molecule, having reached R, will stick within its confines. Direct determination of ψ^* is, of course, very difficult, but in the crystal growth theory of Leubner,[17] it can be expressed as

$$\psi^* = \frac{R^*}{R^* + D/k_+} \tag{8}$$

where k_+ is the rate constant of monomer attachment, a presumed size-independent quantity. It has been determined by Lindfors and co-workers[8] by crystal growth rate measurements on crystallites for which R is on the order of 100 nm.

The customary analytical expressions for ΔG_N are phenomenological and attribute the maximum in this function to the competition between bulk and surface contributions to the free energy, which scale as R^3 and R^2, respectively, where R is an effective radius of the cluster. Since R^3 scales as the volume, which in turn scales as the number of monomers, N, we can write

$$\Delta G_N = -(N-1)k_B T \ln\left(\frac{C_b}{S_0}\right) + \xi\gamma_{sl}(N^{2/3} - 1) \tag{9}$$

where γ_{sl} is an effective solid−liquid interfacial tension, which may depend on N, C_b, the bulk concentration, S_0, the solubility, and ξ, a factor related to the shape and molecular volume of the crystalline material. Lindfors et al.[8] used similar (albeit inconsistent at $N = 1$) equations to fit γ_{sl} against measured nucleation rates.

Forming a cluster of molecules out of an aqueous solution of monomers can be pictured as a three-step process in which first N molecules at some concentration are dehydrated and then brought into contact and arranged in a crystal cluster, and finally, the assembly itself is hydrated. For each of these steps, there is an

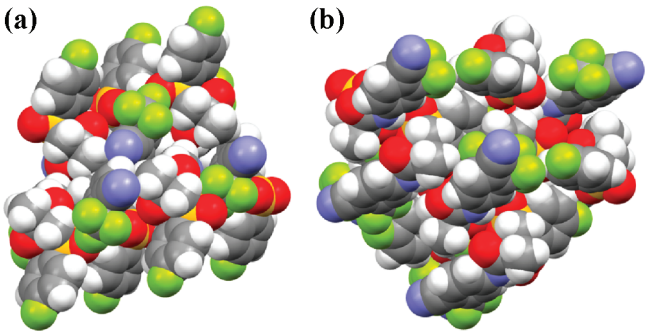

Figure 3. Energy-minimal (a) and radius of gyration-minimal (b) bicalutamide clusters of the monoclinic polymorph ($N = 10$).

associated free energy change. A modified form of eq 9, in which the free energy of clustering has been calculated from these steps, will serve as the basis for the expressed nucleation rate. The remainder of this article will concern itself with this expression.

4. THEORY AND SIMULATION

4.1. Methodology. The problem under consideration poses such a great challenge, both computationally and conceptually, that we are obliged to make a number of approximations to make our calculations tractable while retaining sufficient accuracy for our results to be of practical value. In this initial work, we shall explore a set of semiempirical approximations in an application to clusters of bicalutamide for which experimental data is available or obtained here as described in section 2.

4.1.1. Choice of Clusters. Crystal clusters were chosen by selecting rigid fragment conformations from the bulk crystal lattice. Either of two conditions were used: choosing conformations according to the minimal potential energy or according to the minimal radius of gyration calculated over the geometric center of each molecule. This latter choice was inspired by the findings of Manahoran and co-workers[18] that the preferred packing of small clusters of microspheres is according to the minimum of the radius of gyration. As an illustration of this approach, the energy-minimal decamer of the triclinic polymorph selected is shown highlighted in the crystal lattice in Figure 2. The experimentally determined crystal structure[19] with small adjustments of the bond distances between heteroatoms and hydrogens made according to the recommendations by Allen et al.[20] was used for all crystal clusters. To calculate the energy, no interaction cutoffs were used and the intermolecular interaction terms of the atomically resolved, simple optimized potential for liquid simulation−all atom (OPLSA-AA) molecular mechanics force field[21] with partial charges from the COMPASS27 force field,[22] were summed up to give the total energy.

The energy minimizations of the rigid crystal fragments proceeded through trial-and-error in a stepwise fashion, starting with the dimer fragment. The energy-minimized dimer fragment structure identified, a third molecule was selected from the crystal lattice at random but never more distant than two lattice points away from any other molecule until no further improvement could be detected in the energy of the trimer. This process was then iterated. For the Nth molecule added, the search for further improvement was abandoned after $1000 \times N$ random trial moves failed to lower the energy. The same algorithm was used to find clusters of minimal radius of gyration, substituting the energy calculation by a calculation of the radius of gyration over the geometric centers of all the molecules. In Figures 3 and 4 examples of the decamers for both polymorphs are shown illustrating the resulting cluster geometries according to these two criteria.

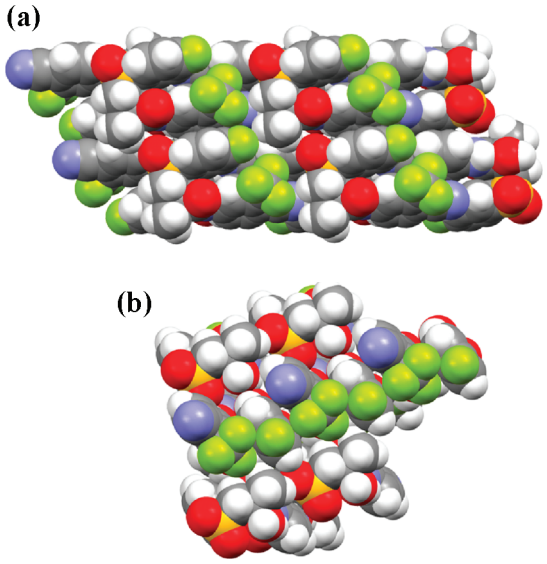

Figure 4. Energy-minimal (a) and radius of gyration-minimal (b) bicalutamide clusters of the triclinic polymorph ($N = 10$).

4.1.2. Simulation Details. All free energies of hydration were computed using the simplified response (SR) theory of Westergren et al.,[1] according to which

$$\Delta G_{\mathrm{hyd}}(N) = \langle E_{\mathrm{LJ}}(N)\rangle + \frac{1}{2}\langle E_{\mathrm{C}}(N)\rangle + \gamma_{\mathrm{vw}}A(N) \qquad (10)$$

in which $\langle E_{\mathrm{C}}(N)\rangle$ is the ensemble average of the Coulomb energy, $\langle E_{\mathrm{LJ}}(N)\rangle$ that of the Lennard−Jones energy, and γ_{vw} the interfacial tension of the vacuum−water interface. $A(N)$ is a measure of the surface area, consistent with the choice of γ_{vw}, of the water cavity in which the solvated cluster is found. This area was computed for a spherical probe of 1.4 Å radius using the Connolly algorithm.[23] However, this choice of surface necessitated a reparametrization of the γ_{vw} value, because it is not identical in magnitude to the one used previously.[1] To this end, the free energy of hydration for a trial set of 39 small organic molecules with their partial charges set to zero was computed using free energy perturbation theory, and the γ_{vw} parameter was calculated thus

$$\gamma_{\mathrm{vw}} = \frac{\Delta G_{\mathrm{hyd}}^{\mathrm{FEP}}(q=0) - \langle E_{\mathrm{LJ}}(q=0)\rangle}{A} \qquad (11)$$

where A is the surface area available to the finite probe, $\Delta G_{\mathrm{hyd}}^{\mathrm{FEP}}$ is the free energy from free energy perturbation theory required to hydrate the zero-charged molecule, and $\langle E_{\mathrm{LJ}}(q = 0)\rangle$ is the average Lennard−Jones energy of such a solute. The details of this procedure are found in ref 1.

For hydration of the monomer, we used 2000 TIP4P molecules and periodic boundary conditions for an isothermal−isobaric Metropolis Monte Carlo simulation[24] at the pressure 1 atm and temperature 298 K with two sets of 2.184×10^8 steps of equilibration and the same number of steps of averaging for two different starting conformations. For reasons to be discussed below, the monomer was kept flexible, i.e., intramolecular degrees of freedom were also sampled. For the clusters, however, only trial moves over

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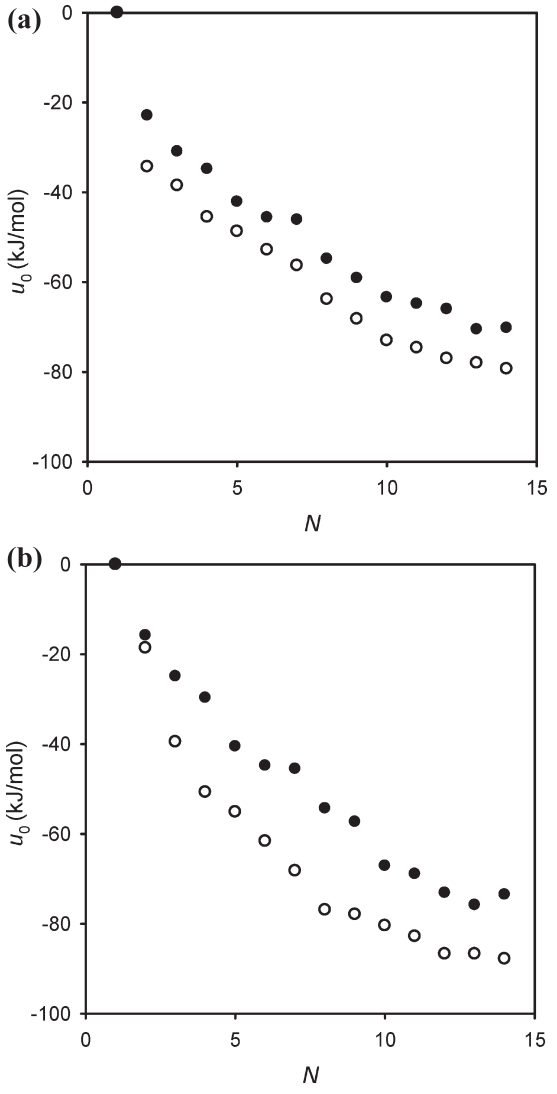

Figure 5. (a) Binding energies per monomer for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the monoclinic polymorph. (b) Binding energies per monomer for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the triclinic polymorph

the positions and angles of the water molecules were used and their number was extended to 9900 with 8.4×10^7 steps of averaging after an equal number of preparatory steps of equilibration. These simulations were performed with the BOSS 4.6 software[25] and used interaction cutoffs of 9 Å for both electrostatic and Lennard−Jones components of the interaction. The electrostatic interaction was forced quadratically to zero over the last half Angstrom. For the Lennard−Jones interaction, a tail correction was applied but no long-range treatment was used for the electrostatics. While arguably the use of the Ewald sum is feasible for these systems, it is not without cost and we believe that the uncertainties in our method are greater than the extra precision possibly afforded by the Ewald sum. Moreover, it should be kept in mind that the force fields we use were optimized without the Ewald sum.

4.2. Results. *4.2.1. Free Energy of Condensation.* Full optimization of the cluster structure by simulation is beyond our capability at the moment. The approximation that the nascent crystallites are built from the bulk crystal structure becomes increasingly accurate in the limit of macroscopic crystallites. It is clear that neither the energy-minimized nor the radius of gyration-minimized conformations necessarily lie on the path passing through the phase space saddle point for crystal nucleation. They are, however, well defined and physically meaningful. The binding energies per monomer, resulting from these calculations, are displayed in Figure 5a and 5b and exhibit a clear tendency to level off at around 14 molecules, beyond which the calculations have not been extended.

It may be safely assumed that the partition function for the clusters is composed of separable internal, q_{int}, and external, q_{ext}, factors; thus

$$Q_{clu} = q_{int} q_{ext} \tag{12}$$

The external contribution is most readily approximated as that of an ideal gas, which should be a passable approximation because the concentration of clusters is expected to be low. Hence, we write

$$q_{ext} = \frac{V}{\Lambda^3} Q_{rot} \tag{13}$$

where $\Lambda = h/(2\pi k_B T N m)^{1/2}$ is the thermal wavelength of the cluster center of mass, V the volume of confinement, m the mass of the monomer, and Q_{rot} the rotational partition function of the whole cluster. We treat the clusters as rigid rotors.

At this point, it is necessary to introduce some model of the internal crystal state of the cluster. We shall assume that the internal vibrations in the monomer remain unchanged as the cluster is formed. Thus, our model shall describe how the translations and rotations of the monomers in the gas phase turn into center-of-mass translation, overall rotation, and relative vibrations in the cluster. We opt for the Einstein model, in which symmetry is present to such an extent that all vibrations have the same frequency, and we write

$$q_{int} = \left[\frac{\exp\left(-\frac{h\nu}{2k_B T}\right)}{1 - \exp\left(-\frac{h\nu}{k_B T}\right)} \right]^{3(N-2)} \exp\left(-\frac{N u_0(N)}{k_B T}\right) \tag{14}$$

in which ν is the frequency of all vibrational modes, h Planck's constant, and $U_0(N)$ the binding energy per molecule of the cluster consisting of $N = 2, 3, \ldots$ molecules. This binding energy was taken from a single-point calculation of the bulk crystal, using the same force field as delineated in section 4.1.1. Six degrees of freedom, corresponding to translation and rotation, have been removed from this expression, as they are accounted for by the external partition function, but otherwise, we assumed that the internal partition function of the bulk crystal (for which there are no external contributions) is the same as the internal contributions of the small cluster. Note that we use the classical point of zero energy so that the ground-state energy of the vibration is $h\nu/2$.

Prior to forming the cluster, we imagine the monomers to constitute an ideal gas of rigid rotors. The total partition function for the whole gas is

$$Q_{gas} = \frac{V^N q_{rot}^N}{\lambda^{3N} N!} \tag{15}$$

where λ is the molecular thermal de Broglie wavelength and q_{rot} the rotational partition function of the monomer, which close to the high-temperature limit may be expressed as

$$q_{rot} = \frac{\sqrt{\pi}}{\sigma} \frac{(k_B T)^{3/2}}{\sqrt{ABC}} \tag{16}$$

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where A, B, and C are the rotational constants of the molecule and σ the number of proper symmetry-equivalent orientations.

We are now in a position to express the free energy change in going from gas to cluster as

$$\Delta G_{gc}(N) = Nu_0(N) - k_B T\left[\ln\left(\frac{N!\lambda^{3N}}{V^{N-1}\Lambda^3}\right) + \ln\left(\frac{Q_{rot}}{q_{rot}^N}\right) + \ln\left(\left[\frac{\exp\left(-\frac{h\nu}{2k_B T}\right)}{1-\exp\left(-\frac{h\nu}{k_B T}\right)}\right]^{3(N-2)}\right)\right] \quad (17)$$

for $N > 1$. To apply this equation, one needs to specify a starting concentration, i.e., the volume and number of monomers contained therein. These are related through $V = N/(\sigma S_0)$, where σ is the supersaturation and S_0 the solubility which we take to be given by the vapor pressure and computed monomer free energy of hydration. The only parameter needed which cannot be calculated outright from the molecular structure or be specified at will is the vibrational frequency, ν. To determine its value, we consider the equilibrium between a bulk Einstein crystal and its vapor. It can be shown that from the equilibrium condition between the ideal gas of rigid rotors and the Einstein crystal one obtains for the vapor pressure the following equation[26]

$$p_{vap}^{Einstein} = k_B T\frac{q_{rot}}{\lambda^3}\left(\frac{h\nu}{k_B T}\right)^6 \exp\left(\frac{u_0(\infty)}{k_B T}\right) \quad (18)$$

with $u_0(\infty)$ taken from a single-point calculation of the bulk crystal; ν was chosen so that eq 18 gives the experimental vapor pressure. The rotational partition function in eq 18 was calculated for the rigid rotor of the elongated "chair" conformation of the bicalutamide molecule.

4.2.2. Free Energy of Hydration. For crystallization in solution, the free energy expenditure of dehydrating the monomers and subsequent hydration of the crystallite must be taken into account. Since this is the only part where the solvent actually makes itself known, it is crucial to predict correctly the free energy differences in crystallization out of different solvents. It was a purposeful choice to keep the monomer flexible to enable future comparisons for nucleation of the two different polymorphic forms of bicalutamide, for which the monomers, even of different conformations in the crystal packing, are indistinguishable in solution. While it might seem inconsistent to keep the clusters rigid and the monomer flexible, it should be kept in mind that this approximation should be less of an issue for large clusters, in which the collective binding energy is dominant. It would doubtless be of benefit to compute the free energies of hydration also for the clusters using exact theory and compare with the predictions of SR theory, but unfortunately the cost of such calculations makes this possibility impractical at present.

The results of the reparametrization of γ_{vw} are displayed in Figure 6 for 39 organic molecules of different type. From these simulations, the average, clearly without trend, of the apparent interfacial tension comes out at 69.0 mN/m if one disgards the smallest molecule data set which is not expected to be of significance in the hydration of the large bicalutamide molecule and even less clusters of it. It should be noted that this value is only slightly greater than 63.5 mN/m, which is the accepted bulk interfacial tension of TIP4P water with respect to vacuum.[27] Hence, we are reassured in our belief that the bulk energy

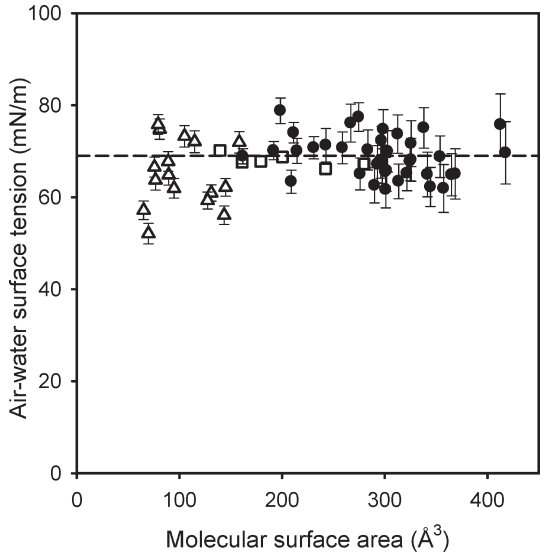

Figure 6. Apparent air−water surface tension for a molecular surface defined by a 1.4 Å Connolly probe computed from free energy perturbation data[4] using eq 11. Open triangles are results for small organic molecules, open squares are results for small alkanes ranging from pentane up to dodecane, and filled circles are results for common drug molecules. The dashed line is the average air−water surface tension obtained for the drug molecules.

contributions to the work of cavity formation are dominated by their surface counterparts. This is clearly reasonable in view of the small size of the cavities. Moreover, we assume that the relative volume changes are negligible for hydration at high dilution, and so we do not discriminate between the Gibbs and the Helmholtz free energies for this process. With the reparametrized γ_{vw}, shown in Figure 7a and 7b are the cluster hydration energy per monomer, $\Delta g_{hyd}(N)$, for clusters up to the decamer of the different polymorphs. Because it is clear that in the limit $N \to \infty$ $\Delta g_{hyd}(N)$ must vanish for consistency, the results presented are still far from reaching the macroscopic limit.

4.2.3. Total Process. The free energy change for the total process of bringing the monomers out of solution into the format of a crystalline, solvated cluster is given as the sum of all individual contributions

$$\Delta G_{tot}(N) = N(\Delta g_{gc}(N) + \Delta g_{hyd}(N) - \Delta g_{hyd}(1)) \quad (19)$$

where $\Delta g_{gc} = \Delta G_{gc}/N$. However, if we consider the limit $N \to \infty$, the bracketed terms in eq 19 turn into $\Delta g_{gc}(\infty) - \Delta g_{hyd}(1)$, which we identify as the chemical potential difference between crystal and dilute solution. If we treat this quantity as a constant with respect to cluster size, we must introduce a correction for the excess free energy in order for the equation to hold true also for finite N. Because the chemical potential difference between the solution and crystal is $-k_B T \ln(C_b/S_0)$, we may also write the CNT-like expression

$$\Delta G_{CNT}(N) = -(N-1)k_B T \ln\left(\frac{C_b}{S_0}\right) + \gamma_{sl}(N)A(N) \quad (20)$$

with $\gamma_{sl}(N)$ being an apparent solid−liquid interfacial tension in this theory and $A(N)$ the surface area of the cluster. Consistency dictates that $\gamma_{sl}(1)A(1) = 0$, and this is also why we subtracted unity from the first factor in the first term. Because these two expressions relate, in principle, to the same quantity, we may

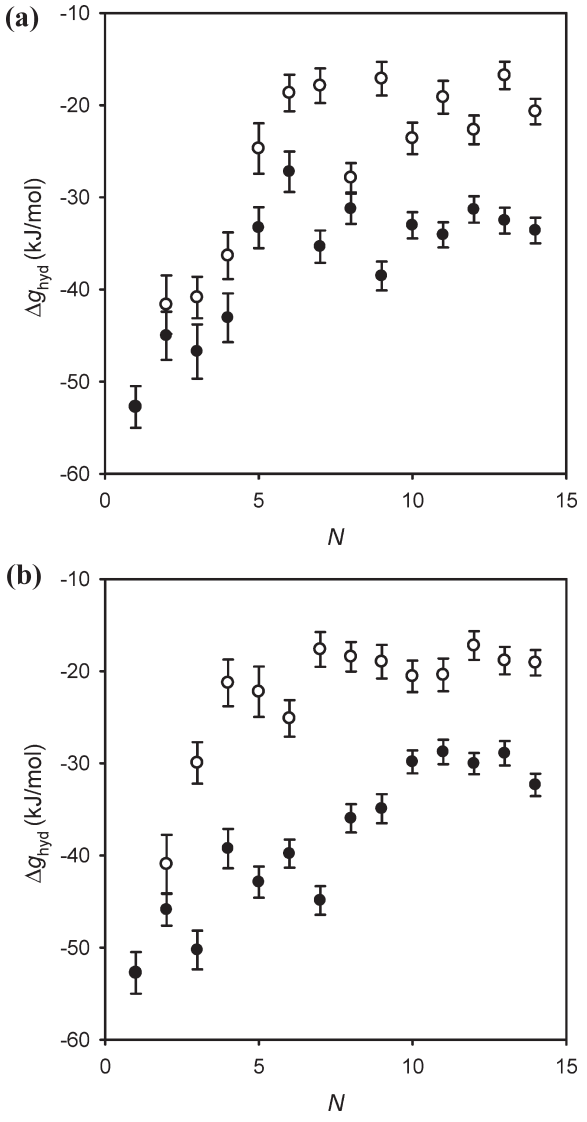

Figure 7. (a) Free energy of hydration per monomer for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the monoclinic polymorph. (b) Free energy of hydration per monomer for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the triclinic polymorph.

solve for the interfacial tension, which is experimentally ascertainable. The algebraic result is shown in eq 21.

$$\gamma_{sl}(N) = \frac{1}{A(N)}\left(\Delta G_{tot}(N) + (N-1)k_B T \ln\left(\frac{C_b}{S_0}\right)\right) \quad (21)$$

It is imperative to point out, however, that the interfacial tension calculated in this way will depend both on the choice of cluster, e.g., shape or monomer count, and also on the precise choice of dividing surface.

The results from eq 21 are shown in Figure 8a and 8b assuming a spherical surface enclosing a cluster volume of density equal to to that in the bulk crystal, i.e., $A(N) = 4\pi((3/4\pi)NV_m)^{2/3}$, with V_m denoting the molecular volume. The spherical surface measure is the most simple and is especially straightforward for comparisons with experiment. The estimated values of the interfacial tensions are 71 ± 5 mN/m for the monoclinic and 68 ± 5 mN/m for the triclinic

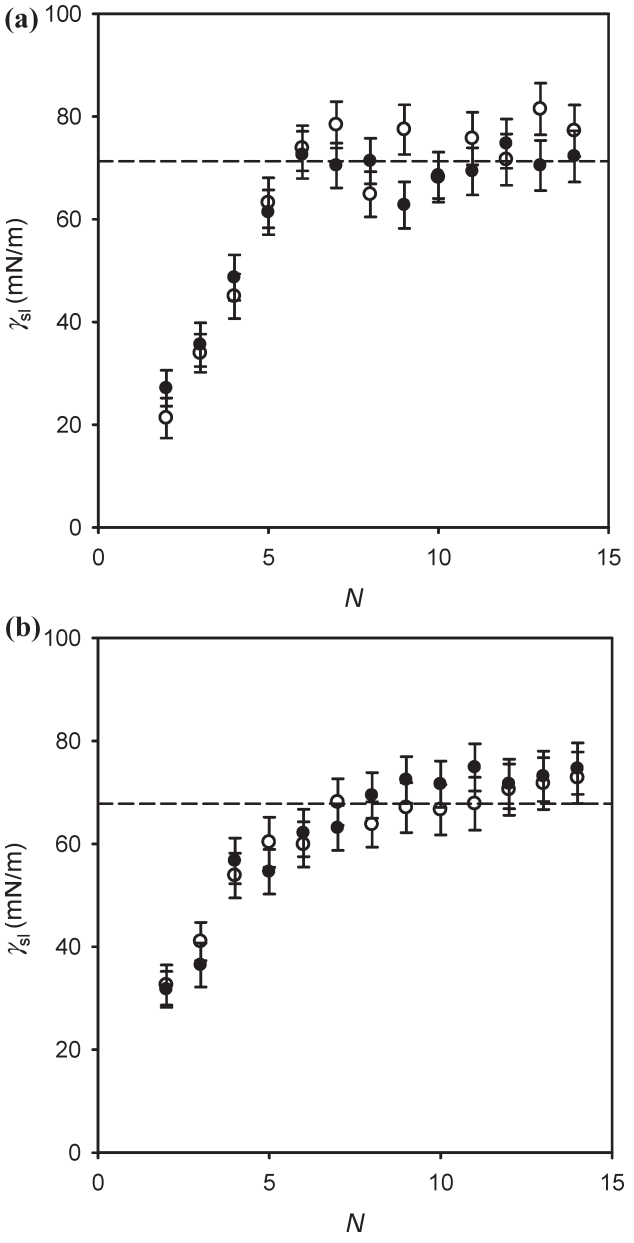

Figure 8. (a) Apparent crystal−water interfacial tensions for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the monoclinic polymorph. The dashed line is the average crystal−water interfacial tension for the monoclinic polymorph obtained for all clusters containing 5−14 molecules. (b) Apparent crystal−water interfacial tensions for energy-minimal (open circles) and radius of gyration-minimal (filled circles) bicalutamide clusters of the triclinic polymorph. The dashed line is the average crystal−water interfacial tension for the triclinic polymorph obtained for all clusters containing 5−14 molecules.

polymorph when the averages are taken for clusters of N = 5, ..., 14 monomers regardless of cluster shape.

5. DISCUSSION AND CONCLUDING REMARKS

The main aim of this paper has been to test a semiempirical methodology for the prediction of drug−water interfacial tension using a simple, molecular mechanics force field, an Einstein model for the crystalline state, the experimental crystal structure,

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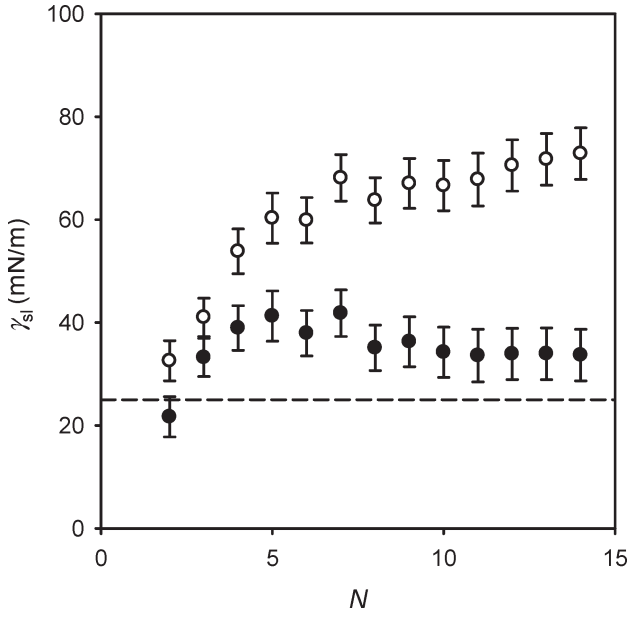

Figure 9. Apparent crystal−water interfacial tensions for energy-minimal bicalutamide clusters of the triclinic polymorph with either uncorrected (open circles) or scaled (filled circles) binding energy and free energy of hydration. The dashed line is the interfacial tension ascertained in crystal nucleation experiments.

the experimental vapor pressure, and regular MC simulations. For the computed surface tensions, comparison with experiment is made difficult because of the paucity of available data. Lindfors and co-workers[8] ascertain the nucleation rate of the triclinic polymorph at constant supersaturation from a nucleation assay method. Interpreting their results in CNT, they extract an interfacial tension between drug and water of 22.1 mN/m. Moreover, they find that it fits in perfectly with an empirical correlation between interfacial tension and solubility derived from Bragg−Williams theory, but it needs to be pointed out that this theory contains two fitting parameters. Moreover, these authors did not use equations for the work of cluster formation that vanish in the monomer limit. Correcting this inconsistency, we carried out work on the original data set for the nucleation experiments and find that, all else being equal, the interfacial tension turns out to be 24.0 mN/m according to the self-consistent classical nucleation theory. This value is further increased to 25.1 mN/m if neglect of the Leubner correction[17] to the integration flux is made. It needs to be made clear also that contrary to the case of a liquid drop the surface tension of a crystal is not unique: each crystal facet has its own thermodynamic characteristics. Hence, when a single value is obtained as in, for instance, nucleation assays or in this work, this value must be interpreted as an effective average.

One inherent flaw in our method is that the Einstein frequency is constant, whereas it is expected to increase with increasing N. This is an entropic effect which should decrease the interfacial tension of the cluster. Not surprisingly, we find that the drug−water interfacial tension seems dramatically overestimated. However, also the crystal binding energy seems to be similarly overestimated by about 50%, and the free energies of hydration are overestimated by about 25%.

These latter findings lead us to investigate to what extent this inherent force field inaccuracy affects the end result. It is possible to empirically correct the drug−drug interaction by scaling by 2/3 and the drug−water interaction by $(2/3)^{1/2}$ to obtain the correct crystal binding energy and free energy of hydration. Because the same scale factor, up to the necessary requirement of the square-root for the Coulomb law and Berthelot mixing rule, can be used for both phases, this is a strong indication that the force field parameters of the bicalutamide molecule alone are responsible for the overestimations in both cases. Such a procedure, with allowance for a new Einstein frequency, produces a much better estimate of the drug−water interfacial tension, as shown in Figure 9 for the energy-minimized clusters of the triclinic polymorph with and without the empirical scaling. The estimated interfacial tension is now 36 ± 3 mN/m for the energy-minimized clusters of the triclinic polymorph for N = 5, ..., 14 and 48 ± 5 mN/m for the energy-minimized monoclinic polymorph. These values still compare very closely to those from the radius of gyration-minimized clusters with the same empirical adjustments, which are 41 ± 3 and 47 ± 4 mN/m for the triclinic and monoclinic polymorphs, respectively. The remaining overestimation of the drug−water interfacial tension is likely due in part to the dependence of the cluster configuration on the aqueous environment, i.e., when selecting clusters to simulate care should be spent on picking conformations favored by the drug−water interaction so that hydrophilic surface domains are dominant over hydrophobic surface domains, and in part to the entropic effect (surface vibrations/relaxation).

For the latter of these, the surface entropic effect, we note that the vibrational modes of the cluster may include modes localized at the surface and modes extending to the surface. Such modes are expected to be shifted to lower frequency in comparison with corresponding bulk crystal modes. Thus, we would expect a more rigorous treatment to yield an Einstein frequency decreasing with decreasing N. Our bulk Einstein model is therefore in error. This error is greatest for the smallest clusters and disappears asymptotically with increasing cluster size. A back-of-the-envelope estimate of its magnitude can be determined assuming all molecules in the cluster are surface molecules. For a simple cubic packing of molecules with only closest-neighbor interactions, such surface molecules may have a vibrational frequency about a factor $2^{1/2}$ lower than the bulk value. Such a rescaling shifts the computed surface tension downward by about 2 mN/m. The most desirable solution would be to numerically compute the work of condensation without empirical input. Future work will hopefully allow us to proceed in this direction.

That the shape of the cluster, as of now decided without any regard to its water interactions, should have an effect on the computed interfacial tensions is obvious in that it cannot escape anyone's notice that the hydration energies per monomer in the clusters exhibit larger variations than their estimated uncertainties. This is especially true of the monoclinic polymorph. The most probable explanation is that the drug surface is very nonuniform with hydrophobic and hydrophilic areas. In building up the cluster, for small clusters especially, the relative abundance of each type varies drastically as the cluster is built up and from here stem the large variations seen in the hydration energies. That this effect is particularly pronounced for the monoclinic polymorph can be explained by the elongated conformation of its monomer. In the triclinic polymorph, the two ring structures of the bicalutamide molecule are folded over each other. In this way, both extrema of the molecule find themselves in close proximity. It follows that if one is more hydrophilic and the other more hydrophobic, however the molecule is turned, both will present themselves to the surrounding water in roughly equal measure.

For the monoclinic polymorph, on the other hand, the molecular conformation is one of complete elongation, thus separating hydrophobic and hydrophilic ends from each other to the maximum extent possible.

That the hydration is a surface-dominated effect is clearly seen in the different hydration energies for the two series of clusters of each polymorph. The clusters minimized with respect to the radius of gyration have a smaller surface area than those minimized with respect to energy. They also have a correspondingly greater magnitude of the free energy of hydration. Hence, the overall hydrophobic nature of the bicalutamide−water interaction is apparent, and it stands to reason that picking clusters with respect to both internal energy and drug−water interactions should bring about a decrease of the computed interfacial tension.

Nevertheless, whereas quantitative agreement is not obtained, it is clear that the qualitative finding that the triclinic racemic form preferentially crystallizes out of aqueous solution is supported by these theoretical data, despite the fact that the energetic ranking of the bulk phase between these two polymorphs is wrongly predicted by the simple force fields that we employed. No doubt, the explanation for the correct ranking of the two interfacial tensions stems from the experimental Einstein frequencies employed. While the inadequacies of a simple molecular mechanics force field for the description of such a complicated molecule as bicalutamide are obvious, the approach outlined in the present paper for estimation of the effective interfacial tensions between the crystal and its solution is still attractive in that the results can be evaluated with respect to different solvents, for which the Einstein frequency, an intrinsic property of the crystal, would be invariant. Moreover, the method is force field independent, and in the future, more powerful computers will allow refined calculations of the quantities entering into the theory.

The very quick convergence of the interfacial tension with cluster size is promising; in the future, a quick calculation of the interfacial tension can possibly be accomplished using a single cluster with $N > 5$. That the interfacial tensions should exhibit such a quick convergence to the macroscopic limit may seem surprising at first glance. Indeed, in their computational study of sodium chloride (NaCl) clusters in contact with the melt at coexistence, Zykova-Timan et al.[28] found that finite-size effects were non-negligible even for clusters of 75 NaCl units or 150 atoms, but it has to be kept in mind that five molecules in the case of bicalutamide crystals constitute a system of 215 atoms and our largest clusters contain 602 atoms. It is at this point very tempting to assume that the electrostatic interactions in the NaCl crystal extend further because of greater electrostatic charges on the atoms.

It is also quite possible that the quick convergence is partly an artifact of the method. That the clusters have not been allowed to relax (e.g., surface melting) may lead to the illusion of quicker size convergence, and this is one of several key aspects of the current work that requires further scrutiny. If the clusters are constrained to be rigid, they share one important aspect with the vacuum cavities computationally studied by Huang and co-workers,[29] namely, the conformational invariance, allowing only the surrounding water molecules to relax. The macroscopic surface tension of water was in their work obtained already around vacuum volumes of 100 Å^3, and as the volumes of these clusters are well above that size already from the outset (the molecular volume is about 500 Å^3 in the crystal of either polymorph), this might explain the quick convergence.

On a somewhat related note, that clusters of very different shapes (see Figure 4a and 4b) produce the same interfacial tension is surprising. However, no deeper significance can at present be attributed to this possibly fortuitous cancellation of free energies of condensation and hydration of the clusters; there is no obvious reason that this would hold to the same extent for any other solvent, as indeed it does not for a vacuum (which can be considered a solvent in this argument).

While the method can beyond doubt be improved, especially concerning the free energy of condensation, a number of possible strong points should be pointed out that could greatly benefit future work if proven. For one, if the quick convergence of the interfacial tensions with cluster size holds up for further scrutiny, it will be computationally very efficient. No more than a single cluster, provided it is sufficiently large, need then be simulated to obtain an estimate of the interfacial tension. While the fortuitous independence of the interfacial free energy on cluster form might not hold for every solvent, it is interesting to investigate to what extent it is applicable for the general case of hydrophobic drug molecules in water, which is the case of main interest in the pharmaceutical industry. As a final remark, if one neglects the free energies of hydration, we can determine also the crystal−vapor interfacial tension. It should be pointed out if one then estimates the contact angle for a water droplet in contact with the crystal powder from the adjusted data, the values obtained, 73 ± 4 for the monoclinic and 71 ± 2 for the triclinic polymorph, may be used for future comparisons with experiment.

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