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Abstract

We investigate the adsorption of insulin onto PE and PVC materials by using HPLC measurements and computer simulations. We interpret the experiments by calculating the Gibbs free energy profiles during the adsorption process. The values of free energy of adsorption show a good agreement with the experimental measurements. The adsorption of insulin onto the different materials is characterized through the conformational changes with respect to its conformation in water and the interfacial regions which are described by specific arrangements of polymer chains, water, insulin and plasticizer molecules.

Keywords

Adsorption, insulin, polymeric materials, potential of mean force, microscopic description, plasticized PVC-TOTM surface, HPLC measurements

1 Introduction

Since medical devices are composed of various polymers and additives, their use for the infusion of drugs should be made with caution. Indeed, interactions between drug, polymers and plasticizers may occur during the delivery process. These interactions can lead to drug sorption which can take the form of an adsorption (surface interaction only) or an absorption (migration into the material). This loss of drug can induce a decrease of the active pharmaceutical ingredient (API) concentration[1–3] and thus lowers the treatment efficiency.[4] Another phenomenon can occur, namely the leaching defined by the migration of the additives into the drug solution affecting thus the product's safety through either the toxicity of leached substances or the degradation of some properties of the drug.[5–8]

A loss of isosorbide dinitrate, tacrolimus, diazepam, amiodarone, insulin has been observed during infusions via PVC tubings.[1,3,8–13] These losses of API by sorption can require an adjustment of the dose administered, based on the expected biological effect of the drug. Flushing the tubing with the infused drug before its administration is a possible way to limit these interactions. This is the case for drugs which are subject to adsorption only like insulin.[12–14] However, for hydrophobic APIs, like diazepam, a simple rinse is not enough. Drug-polymer materials interactions then represents a major issue for the pharmaceutical industry in the delivery of drugs.

Factors affecting drug sorption[9,15] are related to the physicochemical properties of the drug itself (lipophilicity, ionisation state, steric hindrance), the material (amorphous state, added compounds such as plasticizers) and the administration parameters (flowrate, concentra-

tion, pH). Some materials have been proved to be more sensitive to sorption than others. For example, in a recent work[8] it was established that the adsorption of diazepam was less important with polyvinylchloride (PVC) tubings coextruded with polyethylene (PE), polyurethane (PU), thermoplastic elastomer Styrene-Ethylene-Butadiene-Styrene (SEBS) than with PVC. A number of experimental procedures[9,10,15–20] have been developed to determine the drug concentration before/after passing through the medical devices by using HPLC methods to quantify the difference in drug quantities. Nevertheless, the mechanisms at the atomic scale have not been fully understood and it remains difficult to interpret these experiments from the molecular interactions even if experimental procedures focusing on material analyses have also been investigated as an alternative approach.

Very recently, we have shown that a combination of molecular simulations and experiments[21] has made it possible to characterize the sorption of two drugs (paracetamol and diazepam) to pure PVC and PE materials. The adsorption was then characterized through the calculation of potential of mean force and the coupling with experimental measurements provided us a threshold value of about -30 kJ mol^{-1} above which no drug loss was measured. This was a major result on the basis of which we can carry out an interpretation of the experimental data and in the longer term a prediction of the polymer material that prevents sorption.

The challenge taken up here is even more demanding because it is a question of modeling insulin and its adsorption on a plasticized PVC. Indeed, insulin is usually administered by tubings made of PVC and PE polymers. Insulin has been shown to undergo concentration variation due to drug sorption.[1,12] This adsorption then affects the tight glycemic control.[22] Indeed, experiments[1,12] show that the tubings retain variable quantities of infused insulin. Clinicians do adapt their prescriptions to these variations in insulin concentrations by modifying the doses of insulin according to the measured blood sugar (glycaemia) values. However, the causes of these variations can be diverse and it is important to get rid of the contribution of the medical devices on this variability. Having infusion lines that do not interact with insulin could allow the clinician to focus on the pathophysiological origin of the variation in glycaemia without having to consider the impact of the medical devices. Studying these sorption phenomena between insulin and the different constituent materials of infusion tubings is therefore part of a relevant approach to improve the therapeutic management of patients. The impact of this loss during delivery is critical in the neonatal intensive care unit and for long-stay critically ill-patients requiring intensive insulin therapy.[1]

The molecular simulation of insulin is no so widespread[23–28] in the literature because of large system-sizes and corresponding computational resources it requires. The study of the adsorption of insulin onto polymer materials and the calculation of the thermodynamic properties make this study cutting-edge at the limit of what we can do with atomistic models. In addition, we consider a plasticized PVC material for which the modeling of atomistic representative configurations remains an open question.[29–31] To discuss the results of simulations in relation to experiments, we performed experimental studies in conditions of simulating drug uses. Insulin was then put in contact with different tubings (pure PVC, PVC plasticized with tris(2-Ethylhexyl) trimellitate) (TOTM) and PE). The combination of these two techniques should allow the characterization of the interactions between insulin and different materials in terms of energetics and descriptions of the interfaces.

Insulin monomers consist of a globular chain of 51 amino-acids arranged in a A-chain formed by 21 amino acids and a B-chain of 30 amino-acids (see Figure 1). In solution, monomers can form dimers that can also self-assemble to hexamers at high concentrations with zinc ions.[26] Here, we consider the aspart insulin which is an insulin analog in which a proline residue in the B chain at position 28 is replaced with negatively charged aspartic acid. Insulin aspart exists as hexamers that rapidly dissociate into monomers[32] and dimers on subcutaneous injec-

tion. In addition, the native structure of insulin monomers has been shown to be maintained upon adsorption at room temperature.[23] As a result, we will study the monomeric species which is the biologically active form.

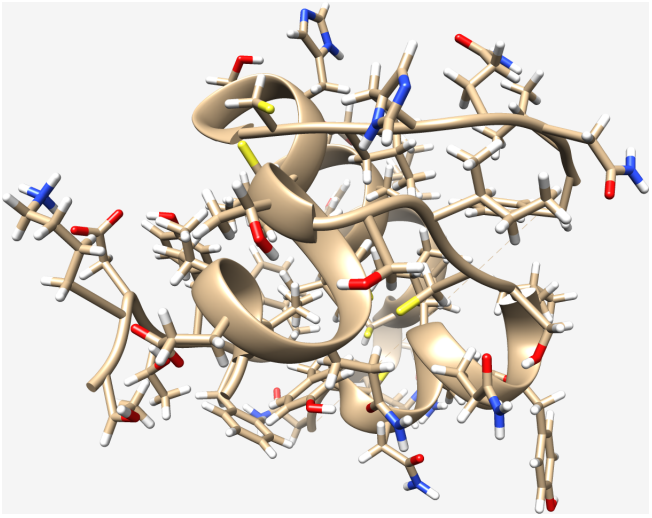

Figure 1: Monomer aspart insulin formed by two peptide chains A and B. Chain A is made up of 21 amino acid residues leading to two α-helices. Chain B contains a central α-helice formed by 30 residues. The two peptide chains are linked by two inter-chains A7(Cys)-B7(Cys) and A20(Cys)-B19(Cys) disulphide bridges (yellow). Another disulphide bridge occurs in the A-chain between A6(Cys)-A11(Cys).

We take the route of investigating adsorption of insulin aspart using both experiments and molecular simulations on three types of polymeric materials unplasticized PVC, plasticized PVC with TOTM and PE. The paper is organized as follows. Section 2 describes the methodological aspects of experiments and molecular simulations. In Section 3 we discuss the main results of this work in terms of free energy profiles along the direction perpendicular to the interface, molecular density profiles and conformations of the adsorbed insulin. Section 4 contains our main conclusions.

2 Materials and methods

2.1 Experimental studies

Insulin loss by sorption was experimentally assessed after contact with PE, PVC and TOTM plasticized PVC.

2.1.1 Materials

The characteristics of the tubings used are presented in Table 1. These tubings were made to order by CAIR LGL (Lissieux, France). 60 mL Polypropylene syringes (Pentaferte, Italy, ref 002022970) were also used.

Table 1: Description of the tubings used in this study in terms of length and inner diameter.

Material	Length (cm)	Inner diameter (mm)
PE	55	4
PVC	42	4
PVC-TOTM	55	4

2.1.2 Active Pharmaceutical ingredients and reagents

Insulin aspart, European Pharmacopeia reference standard was acquired from Sigma-Aldrich SARL (Saint Quentin Fallavier, France) and will henceforth be referred to as insulin. For the chromatographic analysis, acetonitrile (Fisher Chemical, United Kingdom) and formic acid (Fluka, Germany) were used and were certified of HPLC grade.

2.1.3 Methods

Study design Insulin samples were diluted to 3.5 mg mL^{-1} (corresponding to 100 Insulin Unit (IU)/mL) in a sterile 0.9% sodium chloride solution (Fresenius, France). The solutions were diluted with water to reach a final concentration of 1.75 10^{-2} mg mL^{-1} (0.5 IU/mL). Before beginning the experiments, each tubing

was primed with the insulin solution, at a constant flowrate of 1200 mL/h. The priming volume was respectively of 6.9, 5.3 and 6.9 mL for PE, PVC and TOTM/PVC tubings, respectively. Then, each tubing was filled with the insulin solution and stored in a climatic chamber (Binder, model KBF240, Gmbh Tuttlingen, Germany) for 24 hours. Insulin initial concentration was assessed by liquid chromatography at different analytical times : in the rinsing solution (T_0), after a 5 min contact (T_{5min}), at 2 (T_2), 4 (T_4) and 24 hours (T_{24}). At each analytical time, three tubings ($n = 3$) were completely emptied then discarded and the recovered solution was analysed. Insulin loss by sorption was then estimated by comparing insulin concentration at a given analytical time to initial concentration (in the syringe before filling)

Analytical methods Insulin quantification was performed with a liquid chromatography system (LC-2010, Shimadzu) equipped with an UV detector. The stationary phase was an Aeris WIDEPORE XB-C18 (3.6 µm, 100×4.6 mm, 200 Å, Phenomenex, France). The mobile phase was composed of two phases : phase A (water + formic acid 0.1% (v/v)) and phase B (acetonitrile + formic acid 0.1% (v/v)). A gradient was performed over time :

- 0 - 5min : phase A 88%
- 5 - 25 min : phase A 88% → 70 %
- 25 - 30 min : phase A 88%

2.2 Computational procedures

We used the all-atom CHARMM36 forcefield[33] to model insulin, TOTM, PE and PVC molecules. The systems were prepared with the CHARMM-GUI interface.[34] We chose this forcefield because it has shown[33] to perform correctly in reproducing a range of protein NMR properties and in modeling accurately protein structure and dynamics. This force field was optimized using the standard TIP3P model[35] for water. As a result, the water molecules were simulated with the TIP3P model[35] as recommended by some works.[33,36]

Crystalline PE was made of 280 chains of 80 monomers. All the chains were aligned with the y-axis and arranged according to the PE orthorhombic structure with the *Pnam* space group and unit cell parameters (a = 7.40 Å, b = 4.93 Å and c = 2.53 Å). PVC surface was composed of 70 chains of 100 monomers. Plasticized PVC box was composed of 160 PVC chains of 25 monomers and of 308 TOTM molecules. As a result, the plasticizer concentration was equal to 40% (m/m) and correspond to typical concentrations used in medical devices. Insulin structure was obtained from the 4GBN dimeric structure available from the protein databank. A monomeric form was obtained by using the CHARMM-GUI interface. Simulation boxes were created with PACKMOL package[37] and were composed of a polymer surface (PE, PVC or TOTM/PVC), one monomeric insulin, 15000 water molecules and counter-ions (63 Na^+ and 60 Cl^-). The number of chloride and sodium ions were adjusted to both neutralize the system and set the concentration of NaCl to 0.1 M. The box dimensions were L_x= 86.4Å , L_y= 84.1Å, L_z = 90.8 Å. Figure 2 shows a configuration of insulin interacting with a plasticized PVC surface along with conformations of TOTM and insulin molecules.

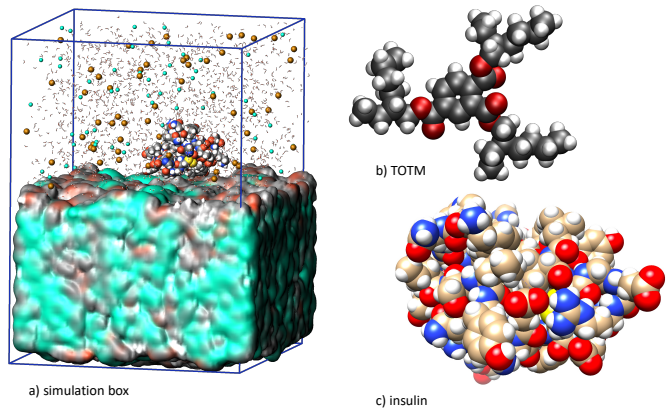

Figure 2: a) Typical configuration of one aspart insulin molecule in interaction with a plasticized PVC including solvent molecules and counter-ions. b) and c) show the chemical structures of the TOTM and insulin molecules.

Molecular dynamic simulations were performed with the LAMMPS package.[38] Periodic

boundary conditions were applied in the three dimensions. The integration of motion was performed by applying the standard velocity-Verlet algorithm with a timestep of 2 fs. The Lennard-Jones crossing parameters were calculated using the Lorentz-Berthelot rules. The Nosé-Hoover thermostat and barostat algorithm were used to maintain the temperature at 300 K and the pressure at 1 atm. The cutoff radius was fixed to 12 Å. The electrostatic interactions were handled with the PPPM 3D method.[39,40]

The potential of mean force (PMF) was calculated by using an extended version (eABF)[41–43] of the Adaptive Biasing Force (ABF) method.[44–47] A description of the calculation of the derivative of the Gibbs free energy profile with eABF is given in a recent paper.[21] The calculations were performed in the Np_{zz}AT statistical ensemble where the surface area $A = L_x L_y$ is constant. In this ensemble, the PMF profile corresponds to the Gibbs free energy profile $\Delta G(z)$ along the z-coordinate between the z-positions of the center of mass of the polymer surface and insulin. Different orientations of the drug with respect to the polymeric surfaces can be sampled by the e-ABF method, as we have shown in a previous paper[21] but it is not guaranteed to observe them over a period of a few hundred nanoseconds. We adopted the convention that the Gibbs free energy $\Delta G(z)$ is zero for the largest separation distance between insulin and the polymeric surface. Statistical fluctuations on the Gibbs free energy profiles were estimated to be in the range of 2-3 kJ mol^{-1} in the region of the Gibbs free energy minimum.[21] These standard deviations were calculated by performing 5 independent calculations of $\Delta G(z)$.[21] The computation of each PMF curve took from 6 to 8 CPU days over 64 processors at a time. Standard molecular dynamic studies were performed by using starting configurations corresponding to those of the Gibbs free energy minimum of the PMF curves. A equilibration time of 1 ns and was followed by an acquisition phase of 20 ns. These simulations were carried out to describe the interfacial region in terms of density profiles and to investigate the possible changes of the conformation of insulin upon adsorption.

3 Results and discussions

We used the HPLC method to detect any loss by sorption of insulin solutions with different tubings. Figure 3 shows the evolution over time (in hours) of the concentration of insulin during static contact with PE, unplasticized and plasticized PVC materials. Even if a drug loss in the range of 3-5% is observed just after filling, we note that this loss reaches after 24 hours 14%, 12% and 8% for PVC, PVC-TOTM and PE tubings, respectively.

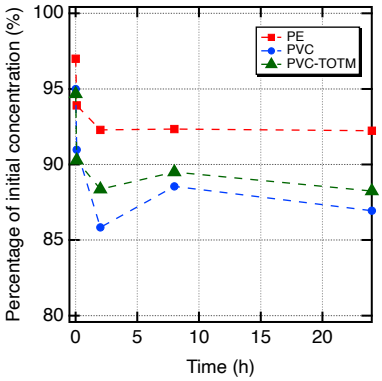

Figure 3: Experimental evolution of insulin concentration in static contact with PE and PVC tubings compared to initial concentrations. The error-bars, less than 1%, cannot be read with the scale used.

These experiments establish that PE and PVC tubings are not completely inert with respect to insulin since they induce a slight loss. As a quantitative comparison reported in a previous work,[21] paracetamol does not show any adsorption with PE and PVC tubings whereas diazepam shows a significant loss of 97% after 24 hours for plasticized PVC-TOTM tubings. In addition, the diazepam loss remains insignificant (less than 1%) for unplasticized PVC tubings and increases to 13% for PE.

We plot in Figure 4 the profiles of the Gibbs free energy $\Delta G(z_1)$ as a function of the z_1-separation distance between the centers-of-mass of the material and insulin molecule. These profiles were calculated with PE, pure PVC and plasticized PVC-TOTM surfaces. These time-consuming PMF calculations show that an adsorption is possible from a thermodynamic viewpoint between aspart insulin and

the different materials. Indeed, the three profiles show portions of curves with negative ΔG values located close to the surfaces indicating the the adsorption process is thermodynamically favored. Let us remind that the calculated free energy of adsorption is a global value including the enthalpy and entropy contributions of both adsorption of insulin and desorption of water molecules initially adsorbed at the surface. The region of lowest negative free energy values extend over a bout 5 Å. The typical values of $\Delta G(z_1)$ in these depths fall into a range of -27 to -21 kJ kJ mol^{-1} as reported in Table 2 as $\Delta G^o_{\rm ads}$ values. These values of $\Delta G^o_{\rm ads}$ are relatively low and describe a weak physisorption mechanism in line with the fact that insulin and the polymeric material interact through weak van der Waals interactions. The interaction between insulin and atoms of the surface is favorable and the electrostatic interactions contribute by 93% to the total energy against only 7% for the Lennard-Jones contribution. The order of magnitude of $\Delta G^o_{\rm ads}$ values is in line with the measurements of loss by sorption of insulin solutions which actually show a slight adsorption of insulin onto tubings. Some configurations representative of the region of minimum free energy are shown in Figure 5.

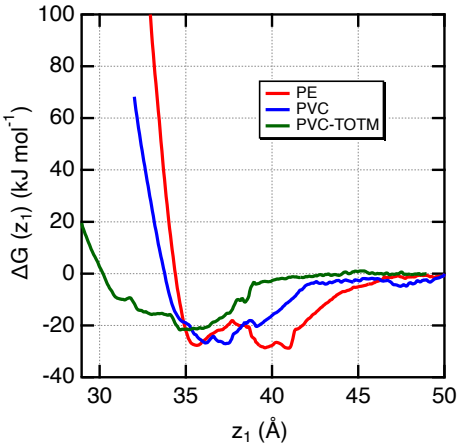

Figure 4: Gibbs free energy profiles $\Delta G(z_1)$ of the adsorption process of insulin onto polymeric materials (PE, PVC and PVC-TOTM) as a function of the z_1-separation distance between the centers-of-mass.

In order to make a comparison with another drug which is known to adsorb onto medical tubings,[19,48] we plot in Figure 6 the PMF curves[21] between diazepam and the same polymeric surfaces. First, the shape of the PMF curves are very different with diazepam : the region of the well of the ΔG curve is much smaller. With the PE surface, the well is very narrow and the width is increasing up to about 2Å with pure PVC and plasticized PVC materials. The width of the well is then explained by the size of diazepam and the nature of the surface. Second, from an energy viewpoint, Figure 6 also shows that the PMF curves calculated with insulin fall between those of diazepam-PVC, diazepam-PE and diazepam-PVC-TOTM interactions for which experiments conclude that there is no loss, slight and dramatic drug loss, respectively. It means that the calculated PMF curves calculated with insulin predict very weak interactions resulting in a moderate adsorption on tubings and a slight loss of insulin.

Another connection with experiments can be made through the calculation of the number of adsorbed molecules. This number is estimated as

$$l_{\rm ads} = \int_{\rm surface}^{\infty} \left(\exp\left(-\frac{\Delta G(z)}{RT} \right) - 1 \right) dz \quad (1)$$

where R is the universal gas constant and T the temperature set at 300K. The number of adsorbed drug molecules per unit area, at equilibrium is the same as the number of such molecules in a slab of solution of thickness $l_{\rm ads}$. Adsorption has a significant impact on the concentration in the solution if $l_{\rm ads}$ is not negligible versus the volume/area ratio of the container, taking into account that roughness actually increases the effective area. The values of $l_{\rm ads}$ are given in Table 2. We observe here that the order of magnitude of this parameter is in line with a slight adsorption of insulin on the tubings investigated here. For a comparison with a drug that leads to a significant adsorption, the interaction of diazepam with PVC-TOTM (see Figure 6) yields $l_{\rm ads} = 4 \times 10^5$ µm. This means when HPLC measurements show a strong adsorption, the values of $l_{\rm ads}$ are by no means comparable (at least two orders of magnitude

higher) to systems with insulin for which only weak adsorptions are detected.

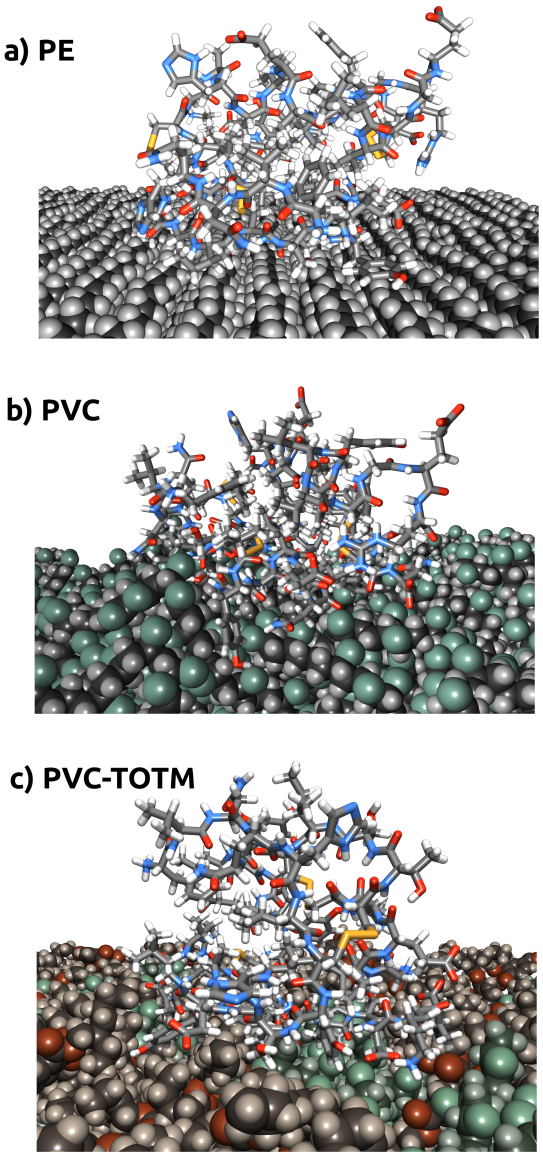

Figure 5: Typical configurations representative of the region of minimum Gibbs free energy for the a) PE, b) PVC and c) PVC-TOTM materials. The water molecules have been omitted for clarity.

Now that the analysis of the PMF curves is in line with the experimental data, we pay attention to the structural insights describing the adsorption of insulin on the different surfaces. The global shape of insulin can be characterized by the asphericity coefficient and the components of the square radius of gyration. The asphericity (δ) was estimated by using the method described by Tsai el al.[49] The eigenvalues α_i were

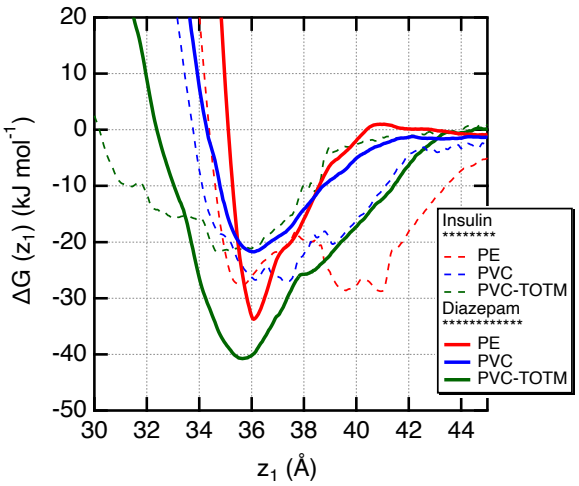

Figure 6: Gibbs free energy profiles $\Delta G(z_1)$ calculated during the adsorption process of insulin and diazepam molecules onto PE, PVC and PVC-TOTM surfaces as a function of the z-separation distance between the centers-of-mass defined here by z_1

obtained from the diagonalization of the radius of gyration tensor and used to calculate asphericity as in Eq.2.

$$\langle \delta \rangle = 1 - 3\langle \frac{\alpha_1\alpha_2 + \alpha_2\alpha_3 + \alpha_1\alpha_3}{(\alpha_1 + \alpha_2 + \alpha_3)^2} \rangle \qquad (2)$$

where <> indicate that the average is carried out over the configurations saved during the course of the simulations. For $\delta = 0$, the insulin adopts a spherical conformation and $\delta = 1$ characterizes a rod-like conformation. The components of the mean square radius $\mathrm{R}g^2_{\alpha,\beta}$ of gyration were also calculated using Eq.3 and listed in Table 2 along with the values of δ.

$$< Rg^2_{\alpha,\beta} >= \frac{1}{M}\sum_{I=1}^{N} m_i(\mathbf{r}_{\mathbf{i},\alpha} - \mathbf{r}_{\mathrm{CM},\alpha})(\mathbf{r}_{\mathbf{i},\beta} - \mathbf{r}_{\mathrm{CM},\beta}) \qquad (3)$$

where m_i the mass of atom i, M the molar mass of insulin and N the total number of atoms. $\mathbf{r_i}$ and $\mathbf{r}_{\mathrm{CM}}$ are the positions of the atom i and the center of mass of insulin, respectively. α and β can represent x, y and z directions. The normal component is calculated via Rg^2_{zz} and the parallel component via $1/2$ ($\mathrm{Rg}^2_{xx} + \mathrm{Rg}^2_{yy}$).

Table 2 indicates that the global shape of the insulin is not fully spherical and characterized

Table 2: Asphericity coefficient δ, number of adsorbed molecules (I_{ads}), normal (Rg^2_{zz}) and parallel ($Rg^2_{para.}$) components of the mean square radius of gyration Rg^2 as a function of the material used. Rg is then calculated as the root mean square radius of gyration Rg^2. Gibbs free energy of adsorption ΔG^o_{ads} are obtained from the minimum of the PMF curves. The subscripts give the accuracy of the last decimal(s), i.e, -27_3 means -27 $\pm$ 3 kJ mol^{-1}. The values in water correspond to a simulation of a free insulin molecule in water.

Device in contact	δ	$Rg^2_{para.}$ (Å^2)	Rg^2_{zz} (Å^2)	Rg (Å)	ΔG^o_{ads} (kJ mol^{-1})	I_{ads} (µm)
PE	0.06_1	43_3	25_2	10.6_{20}	-27_3	24.5
PVC	0.05_1	41_2	21_1	10.1_{20}	-26_3	1.3
PVC-TOTM	0.05_1	35_3	32_2	10.1_{20}	-21_3	8.9
Water	0.05_1	35_8	34_8	10.2_{20}		

by $\delta = 0.05$. The radius of this sphere is about 10.1 Å as suggested by the root mean square radius of gyration. The value of this radius remains unchanged with respect to the different materials and bulk conditions. A thorough analysis requires the calculation of the components of the radius of gyration (see Table 2). When insulin adsorbs onto surfaces, the normal component of the mean square radius of gyration of insulin decreases by up to 21% whereas the parallel component increases by about 22% indicating that the molecule flattens on the surface. We also observe that insulin flattens further with PE and unplasticized PVC surfaces. The presence of the plasticizer mitigates this conformational change with an insulin conformation close to that observed in bulk water. It means that the presence of plasticizers at the surface makes the interfacial region more fluid and weakens the impact of the solid surface.

A contact map is presented in Figure 7 was also computed and represents the distance between each of the amino acids in a 51×51 symmetrical matrix. This contact map is relative to the situation where the insulin is free in water. In other words, Figure 7 informs about the changes in the intramolecular distances with respect to those calculated in water. We observe that the main changes occur in the distances between amino acids belonging to the chains A and B. More precisely, the distances that are modified by adsorption are those that correspond to the distances between amino acids of the A chain (especially the first 10-15 residues) and the last 5 amino acids (Tyr^{26}-Thr^{27}-Asp^{28}-Lys^{29}-Thr^{30}) of the B chain. The ends of both insulin chains are not involved into secondary or tertiary structure and thus present a higher mobility than other segments of the protein. It is therefore not surprising to observe distance variations at the end of the insulin chains suggesting that the overall protein structure was not altered by the adsorption process.

We now focus on the description of the interfacial region in terms of atomic distributions along the normal to the surface (see Figure 8). In the case of PE-water interface, the water density profile shows adsorption peaks close to the crystalline PE surface whereas it decreases steadily to zero in the interfacial region with amorphous PVC surfaces. The atomic density profiles of insulin extend to over a region of about 20 Å in line with the values of radius of gyration. We find again that the density profile of insulin is more extended over z with the plasticized PVC surface in line with the normal component of Rg^2. For the PVC-TOTM surface, the interfacial region is composed of PVC chains, TOTM and water molecules meaning that the insulin interact with all these species in this region. The density profile of water ressembles to that found in liquid-liquid interfaces.[50,51] All these profiles show a physical adsorption of the insulin but the amorphous nature of PVC

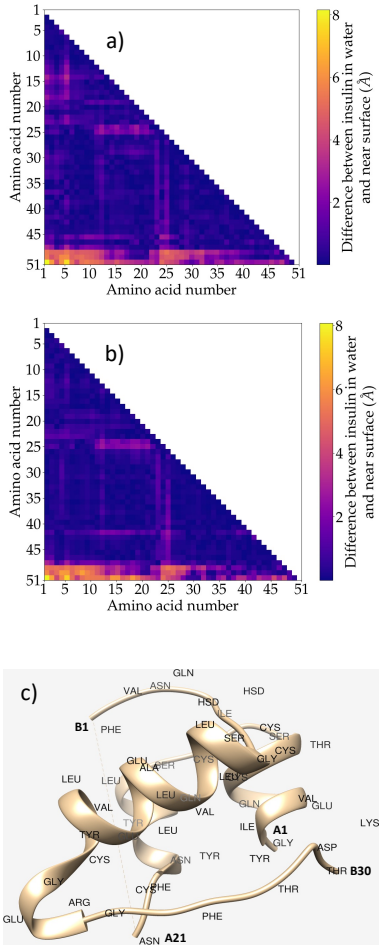

Figure 7: Insulin contact map between the amino acids of chains A and B when insulin molecule adsorbs onto a) PVC and b) PVC-TOTM surfaces. A point of the contact map represents the difference in the distances calculated within the insulin as it is free in water and when it adsorbs onto the material. For completeness, a description of the two chains with a listing of the amino-acids is given in c) where the residues from 1 to 21 are part of the A-chain whereas the B-chain is formed by the residues from 22 to 51.

surfaces makes it possible for insulin to penetrate a little further in the interfacial region without energetically strengthening the adsorption. With the PVC-TOTM surface, the plasticizer molecules sample the PVC surface while forming a layer at the water interface. These density profiles are in line with the features observed in the typical atomistic configurations of Figure 5. With the PE surface, the contact area is on a flat surface whereas we observe local roughnesses for the PVC interfaces showing a conformational adaptation of the adsorbed insulin. Concerning the interaction of insulin with water molecules, we can estimate it through the calculation of the total number of hydrogen bonds between insulin and water molecules. The calculation is based upon the following definition : the X-H..O distance is required to be less than 2.5 Å and the H-X...O angle to be less than 30^o where X represents either O or N. Based upon this definition, we can also calculate the number of intramolecular hydrogen bonds within insulin. In the case of insulin free in bulk water, we calculate a total number of hydrogen bonds of 78 ± 6 of which 19 ± 3 are intramolecular hydrogen bonds. When insulin adsorbs on the PE surface, the total number of hydrogen bonds decreases slightly to 75 ± 6. In the case of the interaction of insulin with PVC-TOTM, the total number of hydrogen bonds including the hydrogen bonds with TOTM molecules decreases to 69 ± 6 with a slight increase of the intramolecular hydrogen bonds (23 ± 3). These calculations show that the loss of hydrogen bonds due to the adsorption is at maximum 11% of the total of hydrogen bonds calculated in bulk water conditions. This analysis confirms that the weak free enthalpy of adsorption is the result of favorable interactions between insulin and polymeric materials through predominant electrostatic interactions and unfavorable free enthalpy contributions due to the desorption of water molecules and dehydration of insulin.

4 Conclusions

In this work, experiments have shown radically different behaviors of drugs (such as for example diazepam and insulin) when they are in contact with PE and plasticized and unplasticized PVC tubings. One way of interpreting these differences in behavior is to look at the microscopic scale to understand molecular interactions. It is then possible by molecular simulations to investigate microscopic effects related

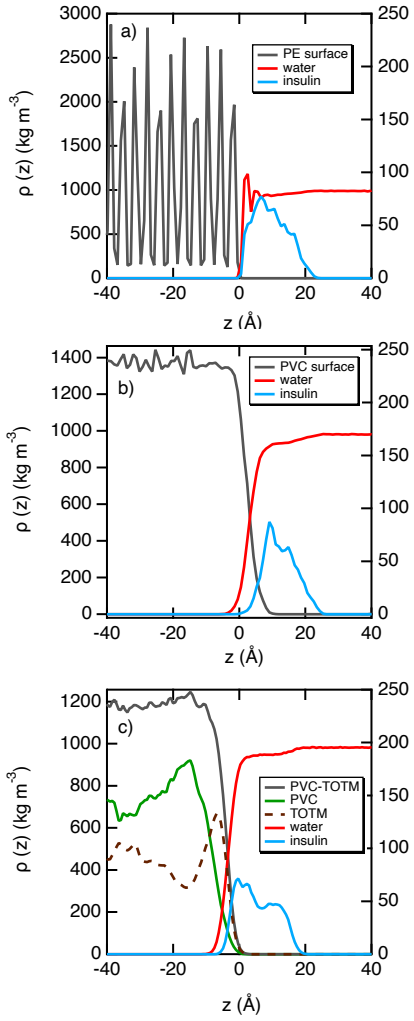

Figure 8: Atomic density profiles of PVC, TOTM, insulin and water molecules calculated in the direction perpendicular to the surface where z represent the position of atoms. The density profiles of insulin should be read on the right-hand scale. The profiles of a), b) and c) represent the adsorption of insulin onto the PE, PVC and PVC-TOTM materials, respectively.

to molecular origins which cannot be seen at macroscopic scales. The statistical thermodynamics takes advantage of linking the microscopic and macroscopic scales and allows us the calculation of the potential of mean force. The interpretation of the experimental results is then done in the light of the calculation of the Gibbs free energy profile. This is not an easy task for insulin and it is already a huge effort to link the two scales. The coupling between experiments and simulations is essential for an in-depth understanding of sorption.

We have combined different molecular simulation methodologies and experiments to investigate the adsorption of aspart insulin on PE, unplasticized PVC and PVC-TOTM materials. HPLC measurements suggest a slight loss of insulin by adsorption but this loss is in no way comparable to that measured with other drugs such as for example diazepam which can reach 97% after 24 hours.[21] The determination of the Gibbs free energy of adsorption through the calculation of the potential of mean forces confirms a weak adsorption which falls into a range of -27 to -21 kJ mol^{-1} for the materials studied here.

Standard molecular dynamics simulations have been performed to characterize the adsorption of aspart insulin in terms of conformational changes and local molecular arrangements at the interfacial region. First, the global shape of insulin can be seen as a sphere of radius 10 Å. The asphericity of insulin is not impacted by the adsorption even if we observe that insulin slightly flattens itself onto the surface. The plasticizer (TOTM) tends to attenuate this effect by making the interfacial region more fluid. The interfacial regions have been analyzed through the atomic density profiles of polymer chains, insulin, water and plasticizer molecules. These simulations have established that the water molecules adopt a different distribution at the interface with PE than with PVC materials. As expected, with the PVC-TOTM surface, the plasticizer molecules remain into the material but also form a layer at the water interface. These profiles have the merit of characterizing the adsorption process at the structural level. A slight loss of hydrogen bonds between insulin and water molecules has been observed upon adsorption.

We have tried to make the simulations as realistic as possible by considering all species (insulin, water, polymer chains and plasticizers). The calculation of the Gibbs free energy profile during the adsorption process remains a genuine challenge as such simulations are time-consuming but the comparison with experiments is very instructive and opens the way for controlling the interactions between drugs and materials to avoid sorption of drugs. These atomistic simulations are essential even on lim-

ited times of a few hundred nanoseconds which prevent us from observing orientation changes, rotations of the molecule and moreover to investigate association/dissociation process through the calculation of residence times of insulin on the surface. All these aspects which imply an evolution in time will only be possible by reducing the number of degrees of freedom and simplifying the model.

In short term, an alternative would be to replace the full atomistic representation of the different species which is computationally prohibitive by a coarse-grained (CG) description of the interactions which should allow to access longer time scales. Nevertheless, these CG simulations need these atomistic simulations with higher resolutions to develop realistic and accurate CG models[52] through bottom-up approaches.[53–55]

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References

(1) Zahid, N.; Taylor, K. M.; Gill, H.; Maguire, F.; Shulman, R. Adsorption of Insulin onto Infusion Sets Used in Adult Intensive Care Unit and Neonatal Care Settings. *Diabetes Res. Clin. Pract.* **2008**, *80*, e11–e13.

(2) Kambia, N.; Dine, T.; Dupin-Spriet, T.; Gressier, B.; Luyckx, M.; Goudaliez, F.; Brunet, C. Compatibility of Nitroglycerin, Diazepam and Chlorpromazine with a New Multilayer Material for Infusion Containers. *J. Pharm. Biomed. Anal.* **2005**, *37*, 259–264.

(3) Maiguy-Foinard, A.; Blanchemain, N.; Barthélémy, C.; Décaudin, B.; Odou, P. Influence of a Double-Lumen Extension Tube on Drug Delivery: Examples of Isosorbide Dinitrate and Diazepam. *PLoS ONE* **2016**, *11*, e0154917.

(4) Tokhadzé, N.; Chennell, P.; Pereira, B.; Mailhot-Jensen, B.; Sautou, V. Critical Drug Loss Induced by Silicone and Polyurethane Implantable Catheters in a Simulated Infusion Setup with Three Model Drugs. *Pharmaceutics* **2021**, *13*, 1709.

(5) Bernarda, L.; Cueff, R.; Chagnon, M.; Abdoulouhab, F.; Décaudin, B.; Breyssef, C.; Kauffmanng, S.; Cosserant, B.; Souweine, B.; for the ARMED study group, V. S. Migration of Plasticizers from PVC Medical Devices: Development of an Infusion Model. *Int. J. Pharm.* **2015**, *494*, 136–145.

(6) Bernard, L.; Bourdeaux, D.; Pereira, B.; Azaroual, N.; Barthélémy, C.; Breysse, C.; Chennell, P.; Cueff, R.; Dine, T.; Eljezi, T.; Feutry, F.; Genay, S.; Kambia, N.; Lecoeur, M.; Masse, M.; Odou, P.; Radaniel, T.; Simon, N.; Vaccher, C.; Verlhac, C.; Yessaad, M.; Décaudin, B.; Sautou, V. Analysis of Plasticizers in PVC Medical Devices: Performance Comparison of Eight Analytical Methods. *Talanta* **2017**, *162*, 604–611.

(7) Bernard, L.; Eljezi, T.; Clauson, H.; Lambert, C.; Bouattour, Y.; Chennell, P.; Pereira, B.; Sautou, V.; on behalf of the ARMED Study Group, Effects of Flow Rate on the Migration of Different Plasticizers from PVC Infusion Medical Devices. *PLoS ONE* **2018**, *13*, e0192369.

(8) Tokhadze, N.; Chennell, P.; Bernard, L.; Lambert, C.; Pereira, B.; Mailhot-Jensen, B.; Sautou, V. Impact of Alternative Materials to Plasticized PVC Infusion Tubings on Drug Sorption and Plasticizer Release. *Sci Rep* **2019**, *9*, 18917.

(9) Jin, S.-E.; You, S.; Jeon, S.; Byon, H.-J.; Hwang, S.-J. Evaluation of Drug Sorption to PVC- and Non-PVC-based Tubes in Administration Sets Using a Pump. *J. Vis. Exp.* **2017**, *121*, e55086.

(10) Treleano, A.; Wolz, G.; Brandsch, R.; Welle, F. Investigation into the Sorption of Nitroglycerin and Diazepam into PVC Tubes and Alternative Tube Materials during Application. *Int. J. Pharm.* **2009**, *369*, 30–37.

(11) Woodward, Z.; Brooks, P.; Morris-Smith, B.; Wallis, M.; Ogbourne, S. M. Adsorption and Leachable Contamination of Flucloxacillin, Cyclosporin and Amiodarone Following Delivery Through an Intravenous Administration Set. *Pharm. Res.* **2018**, *35*, 121.

(12) Hewson, M. P.; Nawadra, V.; Oliver, J.; Odgers, C.; Plummer, J.; Simmer, K. Insulin Infusions in the Neonatal Unit: Delivery Variation due to Adsorption. *J. Paediatr. Child Health* **2000**, *36*, 216–220.

(13) Jakobsson, T.; Shulman, R.; Gill, H.; Taylor, K. The Impact of Insulin Adsorption onto the Infusion Sets in the Adult Intensive Care Unit. *J. Diabetes Sci . Technol. Online* **2009**, *3*, 213–214.

(14) Masse, M.; Maton, M.; Genay, S.; Blanchemain, N.; Barthelemy, C.; Decaudin, B.; Odou, P. In Vitro Assessment of the Influence of Intravenous Extension Set Materials on Insulin Aspart Drug Delivery. *PLoS ONE* **2018**, *13*, e0201623.

(15) Jenke, D. R. Evaluation of Model Solvent Systems for Assessing the Accumulation of Container Extractables in Drug Formulations. *Int. J. Pharm.* **2001**, *224*, 51–60.

(16) Genay, S.; Luciani, C.; Décaudin, B.; Kambia, N.; Dine, T.; Azarouald, N.; Martino, P. D.; Barthélémy, C.; Odou, P. Experimental Study on Infusion Devices Containing Polyvinyl Chloride: To What Extent are They di(2-ethylhexyl)phthalate-free? *Int. J. Pharm.* **2011**, *412*, 47–51.

(17) Hacker, C.; Verbeek, M.; Schneider, H.; Steimer, W. Falsely Elevated Cyclosporin and Tacrolimus Concentrations over Prolonged Periods of Time Due to Reversible Adsorption to Central Venous Catheters. *Clin. Chim. Acta* **2014**, *433*, 62–68.

(18) Ferchichi, M.; Dhaouadi, H. Sorption of Paracetamol onto Biomaterials. *Water Sci. Technol.* **2016**, *74*, 287–294.

(19) Jin, S.-E.; You, S.; Jeon, S.; Hwang, S.-J. Diazepam Sorption to PVC- and non-PVC-Based Tubes in Administration Sets with Quantitative Determination using a High-Performance Liquid Chromatographic Method. *Int. J. Pharm.* **2016**, *506*, 414–419.

(20) Jin, S.-E.; Jeond, S.; Byonb, H.-J.; Hwang, S.-J. Evaluation of Tacrolimus Sorption to PVC- and non-PVC-Based Tubes in Administration Sets: Pump Method vs. Drip Method. *Int. J. Pharm.* **2017**, *528*, 172–179.

(21) Sahnoune, M.; Tokhadzé, N.; Devémy, J.; Dequidt, A.; Goujon, F.; Chennell, P.; Sautou, V.; Malfreyt, P. Understanding and Characterizing the Drug Soprtion to PVC and PE Materials. *ACS Appl. Mater. Interfaces* **2021**, *13*, 18594–18603.

(22) Ley, S.; Ammann, J.; Herder, C.; Hartmann, M.; Kindgen-Milles, D. Variable adsorption of insulin at catheter materials used in intensive care units : polyethylene versus olyurethane - possible cause for hypoglycemia during intensive insulin treatment ? *Crit. Care* **2007**, *11*, 127–.

(23) Mauri, S.; Volk, M.; Byard, S.; Berchtold, H.; Arnolds, H. Stabilization of Insulin by Adsorption on a Hydrophobic Silane Self- Assembled Monolayer. *Langmuir* **2015**, *31*, 8892–8900.

(24) Papaioannou, A.; Kuyucak, S.; Kuncic, Z. Molecular Dynamics Simulations of Insulin: Elucidating the Conformational Changes that Enable Its Binding. *PLoS ONE* **2015**, *10*, e0144058.

(25) Muhammad, E. F.; Adnan, R.; Latif, M. A. M.; Rahman, M. B. A. Theoretical investigation on insulin dimer-β-cyclodextrin interactions using docking and molecular dynamics simulation. *J Incl Phenom Macrocycl Chem* **2016**, *84*, 1–10.

(26) Mukherjee, S.; Mondal, S.; Deshmukh, A. A.; Gopal, B.; Bagchi, B. What Gives an Insulin Hexamer Its Unique Shape and Stability? Role of Ten Confined Water Molecules. *J. Phys. Chem. B* **2018**, *122*, 1631–1637.

(27) Duboué-Dijon, E.; Delcroix, P.; Martinez-Seara, H.; Hladilkova, J.; Coufal, P.; Krizek, T.; Jungwirth, P. Binding of Divalent Cations to Insulin: Capillary Electrophoresis and Molecular Simulations. *J. Phys. Chem. B* **2018**, *122*, 5640–5648.

(28) Soleymani, H.; Ghorbani, M.; Allahverdi, A.; Shojaeilangari, S.; Naderimanesh, H. Activation of human insulin by vitamin E: A molecular dynamics simulation study. *J. Mol. Graph. Model.* **2019**, *91*, 194–203.

(29) Li, D.; Panchal, K.; Mafi, R.; Xi, L. An Atomistic Evaluation of the Compatibility and Plasticization Efficacy of Phthalates in Poly(Vinyl Chloride). *Macromolecules* **2018**, *51*, 6997–7012.

(30) Özeren, H. D.; Olsson, R. T.; Nilsson, F.; Hedenqvist, M. S. Prediction of plasticization in a real biopolymer system (starch) using molecular dynamics simulations. *Mater. Design* **2020**, *187*, 10837.

(31) Özeren, H. D.; Guivier, M.; Olsson, R. T.; Nilsson, F.; Hedenqvist, M. S. Ranking Plasticizers for Polymers with Atomistic Simulations: PVT, Mechanical Properties, and the Role of Hydrogen Bonding in Thermoplastic Starch. *ACS Appl. Polym. Mater.* **2020**, *2*, 2016–2026.

(32) Brange, J.; Ribel, U.; Hanse, J. F.; Dodson, G.; Hansen, M. T.; Havelund, S.; Melberg, S. G.; Norris, F.; Norris, K.; Snel, L.; Sorensen, A. R.; Voigt, H. O. Monomeric insulins obtained by protein engineering and their medical implications. *Nature* **1988**, *333*, 679–682.

(33) Huang, J.; MacKerell Jr, A. D. CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data. *J. Comput. Chem.* **2013**, *34*, 2135–2145.

(34) Jo, S.; Kim, T.; Iyer, V. G.; Im, W. CHARMM-GUI: A web-based graphical user interface for CHARMM. *J. Comput. Chem.* **2008**, *29*, 1859–1865.

(35) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **1983**, *79*, 926–935.

(36) Boonstra, S.; Onck, P. R.; van der Giessen, E. CHARMM TIP3P Water Model Suppresses Peptide Folding by Solvating the Unfolded State. *J. Phys. Chem. B* **2016**, *120*, 3692–3698.

(37) Martinez, L.; Andrade, R.; Birgin, E. G.; Martinez, J. M. Packmol: A Package for Building Initial Configurations for Molecular Dynamics Simulations. *J. Comput. Chem.* **2009**, *30*, 2157–2164.

(38) Plimpton, S. Fast Parallel Algorithms for Short-Range Molecular Dynamics. *J. Comput. Phys.* **1995**, *117*, 1–19.

(39) Hockney, R. W.; Eastwood, J. W. *Computer Simulation Using Particles*; CRC Press, 1988, 1-540.

(40) Pollock, E. L.; Glosli, J. Comments on P3M, FMM, and the Ewald Method for Large Periodic Coulombic Systems. *Comput. Phys. Commun.* **1996**, *95*, 93–110.

(41) Fu, H.; Shao, X.; Chipot, C.; Cai, W. Extended Adaptive Biasing Force Algorithm. An On-the-Fly Implementation for Accurate Free-Energy Calculations. *J. Chem. Theory Comput.* **2016**, *12*, 3506–3513.

(42) Lesage, A.; Lelièvre, T.; Stoltz, G.; Hénin, J. Smoothed Biasing Forces Yield Unbiased Free Energies with the Extended-System Adaptive Biasing Force Method. *J. Phys. Chem. B* **2017**, *121*, 3676–3685.

(43) Fu, H.; Zhang, H.; Chen, H.; Shao, X.; Chipot, C.; Cai, W. Zooming across the Free-Energy Landscape: Shaving Barriers, and Flooding Valleys. *J. Phys. Chem. Lett.* **2018**, *9*, 4738–4745.

(44) Darve, E.; Pohorille, A. Calculating Free Energies Using Average Force. *J. Chem. Phys.* **2001**, *115*, 9169–9183.

(45) Chipot, C., Pohorille, A., Eds. *Free Energy Calculations: Theory and Applications in Chemistry and Biology*; Springer Series in Chemical Physics 86; Springer: Berlin ; New York, 2007; 1-518.

(46) Darve, E.; Rodríguez-Gómez, D.; Pohorille, A. Adaptive Biasing Force Method for Scalar and Cector Free Energy Calculations. *J. Chem. Phys.* **2008**, *128*, 144120.

(47) Comer, J.; Gumbart, J. C.; Henin, J.; Lelievre, T.; Pohorille, A.; Chipot, C. The Adaptive Biasing Force Method: Everything You Always Wanted to Know but Were Afraid To Ask. *J. Phys. Chem. B* **2015**, *119*, 1129–1151.

(48) Salloum, H. A.; Saunier, J.; Aymes-Chodur, C.; H. Barakat, N. Y. Impact of the Nature and Concentration of Plasticizers on the Ability of PVC to Sorb Drug. *Int. J. Pharm.* **2015**, *496*, 664–765.

(49) Tsai, C.-F.; Lee, K.-J. A Comparative Study of the Second-Order Hydrophobic Moments for Globular Proteins: The Consensus Scale of Hydrophobicity and the CHARMM Partial Atomic Charges. *Int. J. Mol. Sci* **2011**, *12*, 8449–8465.

(50) Ndao, M.; Devemy, J.; Ghoufi, A.; Malfreyt, P. Coarse-Graining the Liquid-Liquid Interfaces with the MARTINI Force Field : How is The Interfacial Tension Reproduced ? *J. Chem. Theory. Comput.* **2015**, *11*, 3818–3828.

(51) Ghoufi, A.; Malfreyt, P.; Tildesley, D. J. Computer Modelling of the Surface Tension of the Gas-Liquid and Liquid-Liquid Interface. *Chem. Soc. Rev.* **2016**, *45*, 1387–1409.

(52) Vaiwala, R.; Ayappa, K. G. A Generic Force Field for Simulating Native Protein Structures Using Dissipative Particle Dynamics. *Soft Matter* **2021**, *17*, 9772–9785.

(53) Maurel, G.; Schnell, B.; Goujon, F.; Couty, M.; Malfreyt, P. Multiscale Modeling Approach toward the Prediction of Viscoelastic Properties of Polymers. *J. Chem. Theory. Comput.* **2012**, *8*, 4570–4579.

(54) K.Kempfer,; Devémy, J.; Dequidt, A.; M.Couty,; P.Malfreyt, Realistic Coarse-Grain Model of cis-1,4-Polybutadiene: From Chemistry to Rheology. *Macromolecules* **2019**, *52*, 2736–2747.

(55) K.Kempfer,; Devémy, J.; Dequidt, A.; M.Couty,; P.Malfreyt, Development of Coarse-Grained Models for Polymers by Trajectory Matching. *ACS Omega* **2019**, *4*, 5955–5967.

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