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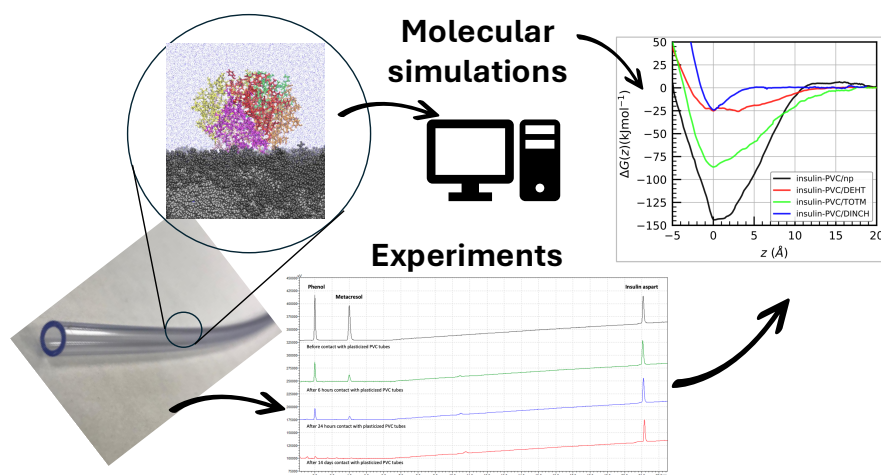


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Graphical Abstract

Interactions of insulin aspart hexamer and excipients with plasticized polyvinyl chloride surfaces: a comprehensive investigation combining molecular simulations and experiments

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Highlights

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- Insulin aspart hexamer adsorption onto plasticized and non-plasticized PVC surfaces is thermodynamically favourable
- The presence of plasticizers modifies the solid-liquid ionic interface without reducing insulin aspart hexamer adsorption
- The adsorption of phenolic excipients of insulin aspart is increased for plasticized PVC
- A macroscopic diffusion model reproduces the losses by sorption of phenolic excipients by using properties calculated from molecular simulations.

Interactions of insulin aspart hexamer and excipients with plasticized polyvinyl chloride surfaces: a comprehensive investigation combining molecular simulations and experiments

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Abstract

Insulin aspart is a major therapeutic biomacromolecule used worldwide for the treatment of diabetes mellitus. It is administered either subcutaneously or intravenously, using infusion lines made most often from plasticized polyvinyl chloride (PVC). Unfortunately, its very nature makes it at high risk of surface interactions with the materials it can come into contact with, leading notably to greatly decreased concentrations and patient underdosing. In order to prevent this phenomenon, for which no adequate solution yet exists, in-depth knowledge of the behaviour of insulin aspart at the water-solid interface is needed. The aim of this work was to shed new light on this highly problematic interaction and explain the adsorption phenomenon of insulin aspart and its phenolic excipients (phenol and metacresol) to plasticized PVC tubings from a thermodynamic point of view by combining experimental and molecular dynamics simulations. Our results proved that the hexameric form of insulin aspart possesses an important affinity for the PVC surface, to which it adsorbs nearly instantaneously to, whilst phenol and metacresol interacts with the PVC / plasticizer interface of the material. The molecular simulations of these surface interactions correlate well with the sorption processes that can be assumed to happen with the negatively charged PVC surfaces. Thermodynamic values obtained via the molecular simulation process were used to model successfully the experimental data. This combination of theoretical approaches could help us in predicting the risk of interfacial interactions in biomacromolecular systems.

Keywords: Molecular simulations, Sorption, Molecular description, Adsorption of insulin aspart hexamer, Absorption of phenolic excipients, Free energy calculations, Macroscopic diffusion model

1. Introduction

Interactions between biological therapeutics and material surfaces can take on many forms and have varied consequences, such as the binding (adsorption) of active substances to the materials they come in contact with. The term "adsorption" generally means the accumulation at an interface (liquid-solid, liquid-vapour or vapour-solid) of atoms or molecules coming from one of the phases. These phenomena are commonly classified as being either of the domain of physisorption (corresponding to reversible, small energy bindings, mainly of Van der Waals origins) or of the domain of chemisorption (with large binding energies, of either ionic or covalent nature)[1]. Initially covering the domain of low molecular weight compounds (such as gases), the scientific concept was quickly expanded to more complex molecules, such as biomolecules (including proteins), for example during the study of biological fluids and their interactions with various materials and in diverse domains [2, 3].

Although protein adsorption in itself has been studied for many years now, [4, 5], even recent studies using molecular

dynamics [6] or machine learning [7] do not allow a complete understanding of all its intricacies. This applies to protein adsorption in both a biological context (plasma proteins onto implanted biomaterials for example) and in a pharmaceutical or clinical context, during which the protein (hormone, monoclonal antibody (mAb) or genetic material) can interact with various container or administration device materials [5, 8, 9, 10, 11, 12]. These interactions can notably cause a loss of active pharmaceutical ingredient (API) leading to potential patient underdosing, and it has even been reported that they could impact the translatability of preclinical safety assessments [13]. Out of the peptides most studied, insulin and its analogues like insulin aspart stand out in particular. Insulin aspart is a rapid-acting, human insulin analog that is used worldwide to improve glycemic control for treating type 1 and type 2 diabetes mellitus in adults and children. It is a protein hormone, composed of 2 chains (A and B), and acts by increasing the cellular penetration of glucose. It is structurally nearly identical to human insulin, except for the replacement of a single proline amino acid at position 28 in the C-terminal area of the insulin B-chain with a negatively charged aspartic acid residue [14], and thus possesses a molecular weight of 5826 Da. Once in the human body it is biologically active in a monomeric form, but is stabilized for long term storage in an hexameric

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form (which is a nearly 35 kDa complex) by the use of zinc and phenolic compounds (phenol and metacresol), and encapsulates 10 water molecules in a barrel like canal, withholding 2 Zn^{2+} ions at each extremity [15, 16]. Whilst its therapeutic efficacy has been demonstrated throughout numerous clinical studies, its proteic nature makes it especially vulnerable to adsorption which can occur during all the stages of its production, storage or clinical use. Most problematically, this can happen during its intravenous infusion into critically ill patients hospitalized in adult or pediatric intensive care units [17], and can result in certain cases in up to 50% of the intended insulin not being delivered over 24 hours in a low flow and low concentration context [18]. This loss of insulin, which can have dramatic clinical consequences in certain cases, has been noted by various authors in different conditions of insulin (and its analogues) concentrations, flow rate and infusion tubing material (i.e infusion lines and catheters) used to deliver the drug to the patients (such as but not exhaustively: [19, 20, 21]). All this data adds up to a simple assessment: insulin administration remains an at-risk situation, for which no obvious solution has yet been developed. Whilst some authors have recommended priming or rinsing the infusion lines before use on the patient [22, 23], this practice only brings limited security. It seems therefore paramount to be able to better understand the intrinsic mechanistic interactions occurring at the water-solid interface between the insulin aspart medication and the infusion lines made generally of plasticized PVC in order to be able to one day either develop a material that would be inert towards insulin and not promote its adsorption, or find an optimum galenic formulation that would prevent these interactions from happening. As the intricacies of interactions at the molecular level are not easily reachable using experimental methods, molecular simulations have recently shown themselves to be useful tools to gain additional knowledge about the phenomena happening at such a small scale. Indeed, some studies have already been carried out on insulin using molecular simulations. The interaction between an hexamer of insulin and one of its phenol ligand was investigated by using both free energy techniques and molecular dynamics simulations [24]. The description of the conformation of human insulin dimer [25], the conformation changes of insulin [26], the role of water in stabilizing mutant insulin dimers [27], the binding of different divalent cations with the insulin monomer [28], the shape of the insulin oligomers and the role of water molecules [16, 29], the impact of arginine on insulin oligomerization [30], the interaction of vitamins with human insulin monomer [31], the albumin-insulin detemir association [32] are all issues that have been tackled by using atomistic simulations and specific methodologies for the calculation of free energy [33]. A recent study [34] used a polarizable model to investigate the structure-function relationship of insulin monomers. In a previous study our team also investigated the adsorption of the insulin aspart monomer onto tubes of PVC plasticized with tri-(2-ethylhexyl) trimellitate (TEHTM, also known as TOTM)[35], however no data exists about the molecular interactions between the hexameric form and plasticized PVC surfaces, as well as potential interactions of the phenolic excipients (phenol and metacresol) with

the tubings surfaces, despite the fact that the compounds have been shown experimentally to play a huge role in insulin aspart stabilization and that any loss could be detrimental to its stability [36, 37]. Indeed, the formulation and stability of biological macromolecules cannot be envisioned without taking into account the excipients, as far from being “inactive” ingredients in pharmaceutical formulations they often play a critical role. Indeed, all commercialized insulin formulations contain phenolic excipients (phenol and/or metacresol) as well as zinc oxide [38]. Phenol and metacresol even possess a dual role as they both are antimicrobial preservatives (and are thus needed to help preserve the sterility of the injectable insulin medication) and also actively physicochemically stabilize the insulin hexamer form through hydrogen bonding [39, 40]. Teska *et al.* even showed that insulin aspart was highly sensitive to phenolic preservative concentrations as it had a decreased stability when depleted of preservatives, even over short periods [41]. Therefore, maintaining adequate concentrations of these important compounds at all stages of the drug’s life cycle (production, conditioning, storage, administration) is fundamental to be able to maintain its final clinical efficacy, and even manufacturers struggle with losses caused by interactions of phenol and metacresol with polymer tubings [36, 42].

The objective of this study was therefore to shed new light on an highly problematic interaction and explain the adsorption phenomenon of insulin aspart to plasticized PVC tubings from a thermodynamic point of view by combining experiments and molecular simulations. To do so we applied intensive molecular simulations of the insulin hexamer to study its adsorption process onto plasticized PVC surfaces by using the calculation of the free energy profile along the separation distance perpendicular to the surface. We gave molecular insights about the hexamer during adsorption in terms of distributions of partial charges, number of hydrogen bonds with water and global structure. We also investigated the absorption of phenolic excipients by developing alchemical transformations and we used some data obtained from these molecular simulations in a macroscopic diffusion model to successfully reproduce the losses of excipients.

2. Materials and methods

2.1. Experimental protocol

The loss by sorption of insulin aspart, phenol and metacresol was experimentally assessed during 24h of static contact of the diluted insulin aspart medication with different types of PVC: non-plasticized (np), and plasticized with di(ethylhexyl) terephthalate (DEHT), 1,2-cyclohexanedioic acid diisononyl ester (DINCH) and TOTM.

2.1.1. Materials and compounds

Insulin aspart (henceforth referred to as insulin) was purchased in the form of the 100 UI/mL (3.5 mg/mL), 10 mL Novorapid® medication vial (from NovoNordisk, France). Sterile 0.9% saline solution was procured from Fresenius Kabi

(France). The analytical standards that were used were the following: Insulin aspart, European Pharmacopeia reference standard (reference Y0000349), phenol (reference 16016-100G-R) and metacresol (reference 65996-5ML-F) and were acquired from Sigma-Merck (Saint Quentin Fallavier, France). For the chromatographic analyses, acetonitrile (Fisher Chemical, United Kingdom) and formic acid (Fluka, Germany) were used and were certified of HPLC grade.

The PVC tubings that were used were made to order by CAIR LGL (Lissieux, France) and had the following characteristics: length 50 cm, interior diameter 4 mm, and mass of plasticizer varying from 30% (DEHT) to 37 % (TOTM) m/m [43]. Plastipack 50 mL polypropylene syringes with luerlock (reference 300865 from Becton Dickinson, France) were also used.

2.1.2. Study design

The insulin solution was diluted in a 0.9% sodium chloride solution (Fresenius Kabi, France) to a concentration of 0.5 UI/mL (corresponding to $1.75 \cdot 10^{-2}$ mg/mL) which is commonly used in clinical practice. Each tubing was filled with the diluted insulin solution using a syringe pump (Ochestra DPS syringe pump, from Fresenius Kabi) at a constant flow rate of 1200 mL/h, then plugged. The tubings were then stored at 25°C and 60% humidity in a climatic chamber (Binder, model KBF240, GmbH Tuttingen, Germany) for times ranging from 15 min to up to 24h. At the end of each allotted contact time (15 min, 30 min, 60 min then 2h, 4h, 8h, 24h), three tubings ($n = 3$) were fully emptied of their content by gravity, the contents recovered and immediately transferred into glass chromatography vials and analysed by liquid chromatography. Insulin, metacresol and phenol losses by sorption were then expressed by comparing the quantified concentration at a given analytical time to the initial concentration (in the syringe before filling).

2.1.3. Compound quantification

Insulin, phenol and metacresol were quantified using a high pressure liquid chromatography (HPLC) system (LC-2010-HT, Shimadzu, France) equipped with a UV-visible detector, following a previously published method [44, 35]. Briefly the stationary phase that was used was an Aeris Widepore XB-C18 reference 00D-4482-E0 (dimensions 100X4.6 mm, 3.6 μ m particle size, 200 Å pore size), from Phenomenex (France). The mobile phase was made up of a gradient mixture of phase A (water + 0.1% formic acid, v:v) and phase B (acetonitrile + 0.1% formic acid, v:v). The flowrate was of 1.3 mL/min and the gradient parameters were the following:

- 0 – 5 min: phase A 88%, phase B 12%
- 5 – 25 min: phase A 88% → 70% and phase B 12% → 30%
- 25 – 25.1 min: phase A 70% → 88% and phase B 30% → 12%
- 25 – 30 min: phase A 88%, phase B 12%

The oven temperature was of 30°C, and the quantification wavelength was set at 210 nm. The samples were injected without additional dilution (theoretical insulin concentration of 0.5 UI/mL). The quantification method had been previously validated with a target mean repeatability, intermediary fidelity and exactitude bias coefficients of less than 5%. More specifically, the repeatability coefficient at 0.5 UI/mL was of 1.58%, and the exactitude bias coefficients between 0.3 and 0.55 UI/mL (60% to 110% of the target concentration) were of maximum 0.45%, thus allowing precise quantification of insulin in this setting. Any variation above 2% can be considered as indicative of a significant change in concentrations.

2.1.4. Surface zeta potential measurements

The electrokinetic potential measurements of flat surfaces (non plastified PVC, PVC/TOTM, PVC/DINCH and PCV/DEHT plates; 5 samples of each were studied) were performed with a SurPASS3 instrument (Anton Paar GmbH, Graz, Austria) using the streaming potential technique. The measurements were performed with the clamping cell, a cell designed for the analysis of solid samples with a flat surface and rectangular shape with an analysis area of 20 × 10 mm. The distance between the sample surfaces and the polypropylene reference was adjusted to $100 \pm 5 \mu$ m. A 0.001 M KCl solution was used as electrolyte.

For the study of the isoelectric points (IEP) of the polymer plates, we used the pH dependence of the zeta potential in the pH range from 9.5 to ≈ 2.5 (positive value closest to the IEP). The pH was adjusted using 0.05 M NaOH and 0.05 M HCl solutions.

The 0.001 M KCl solutions (Potassium chloride, Fluka, Honeywell) were prepared with the VWR Ultrapure water systems (From VWR International, resistivity at 25° C = 18.2 M Ω .cm).

Before performing the measurements, the samples were rinsed 3 times with the electrolyte and the Gap Height was set to $100 \pm 5 \mu$ m. The solution was then changed and 5 zeta potential measurements were performed and the average zeta potential was determined using the following Helmholtz-Smoluchowski relation:

$$\zeta = \frac{dU_{str}}{dp} \frac{\eta}{\varepsilon \varepsilon_0} \frac{L}{A} R \quad (1)$$

where U_{str} is the streaming potential, dp is the applied hydraulic pressure, η is the viscosity of the liquid, ε is the permittivity of the liquid, ε_0 is the permittivity of vacuum, R is the electrical resistance across the medium and L , A are the length and cross-sectional area of the channel, respectively. During the measurements, the electrolytic solution was continuously purged with nitrogen at 5.5 bar. A pressure gradient of 200 to 600 mbar was applied to generate the streaming potential. For the determination of the IEP, 4 zeta potential measurements were performed at each individual pH. The pH variation between 2 measurement points was of maximum 0.3 pH units. Before performing the pH measurement, two rinsing cycles with the electrolyte were performed.

All experiments were performed at 25° C \pm 5° C. The pH of the solutions was measured by an Ag/AgCl reference elec-

trode that was regularly calibrated using standard buffer solutions ($pH_{buff} = 3, 7, 10$, from Hanna Instrument). The conductivity electrode was calibrated using a 0.1 M KCl solution. Both electrodes were calibrated using the SurPass software following the manufacturer's instructions.

2.2. Simulation Protocol

The all-atom CHARMM36 force field [45] was used to simulate insulin aspart (also referred to only as "insulin"), excipients (phenol and metacresol), ions (sodium and chloride), PVC polymer, and plasticizers molecules. The crystallographic structure of the insulin was obtained from the protein databank, (PDB code: 1ZEH[46]). This structure (which was obtained by X-ray diffraction method with a resolution and R-value of 1.5 angstrom and 0.193, respectively) contained 15 molecules of metacresol for the hexamer. To obtain the complete hexameric structure, we applied symmetry operators corresponding to the space group H3: (X, Y, Z), (-Y, X-Y, Z), and (-X+Y, -X, Z) to the dimer coordinates. The water model used was the TIP3P model [47]. The systems were prepared using the CHARMM-GUI interface [48]. The PVC and the plasticized PVC slab were composed of 200 PVC chains of 35 monomers. For the plasticizer concentration in all cases it was equal to 37% (m/m) as in previously published research [43]. The polymer slabs were prepared separately from the liquid phase. The PVC chain with the plasticizer molecules solid phase was equilibrated alone with a heating-cooling procedure (300K-800K-300K) at a rate of 50K ns⁻¹. There were around 30,000 water molecules in each system with 86 chloride (Cl⁻) and 100 sodium (Na⁺) ions, to neutralize the system and set the concentration of NaCl to 15 mmol L⁻¹ (equivalent to a 0.9% saline solution). The box size for the insulin and the water molecules were initially set to 100 angstrom. The slab of polymer for the non plasticized PVC was $L_x = L_y = 100 \text{ \AA}$ and L_z about 50 \AA . For the plasticized PVC the aqueous phase was the same and the polymer still composed of 200 chains of 35 monomers plus a plasticizer phase composed of 658, 605 and 470 molecules of DEHT, DINCH and TOTM, respectively. Molecular dynamics simulations were performed using the GROMACS package [49]. The potential of mean force (PMF) curves or free energy profiles along a specific coordinate [50, 51, 52] were calculated using the umbrella sampling method [53]. A time-step of 1.0 fs was used, with periodic boundary conditions in all directions. An energy minimisation was performed using a steepest descent algorithm for energy minimization with a convergence criteria of 750.0 kJ.mol⁻¹.nm⁻¹ for the maximum force. Then an equilibration simulation in the NPT ensemble was performed, with the first 10 ns at 60K, followed by a temperature rise to 300K for 100 nanoseconds and finally 100 nanoseconds at 300K. The C-rescale barostat with time constant of 2.0 ps and the V-rescale thermostat with time constant for coupling of 0.1 ps were used. The Lennard-Jones and coulomb interactions were set to have a cutoff of 10 angstroms, the Particle Mesh-Ewald (PME) was used for long range electrostatics interaction and the LINCS algorithm was used to handle constraints off the H-bonds. The acquisition on which the analyses (density and

charges profiles,...) were performed lasted for 200 ns. To compute each PMF curve, 150 independent simulations were carried out, each consisting of 2.0 ns of equilibration followed by 5.0 ns of data acquisition. These simulations were uniformly distributed along the molecular reaction pathway. The initial configurations for each simulation were obtained from a prior pulling simulation, in which a constant force was applied along the z-axis of the simulation box, using a spring constant of 4000 kJ mol⁻¹ nm⁻² and a pulling rate of 0.01 nm ps⁻¹. For further details on the protocol, the reader is redirected to ref.6.

Free energy potentials (FEP) [54, 55, 56, 33] were measured for systems composed of 25 chains of 75 monomers of PVC plus 10 molecules (for the MSD calculations) or just 1 molecule (for the FEP calculation) of excipients (metacresol or phenol) plus depending on the plasticizer 143 molecules of DEHT, 131 molecules of DINCH, 102 molecules of TOTM (the number of plasticizer molecules was chosen so as to reach a 37% mass of plasticizer). The FEP simulations were run in quintuplicate (n = 5). The MSD simulations were performed on one system during 100 ns with the configurations saved every 5 ps. All systems were equilibrated the same way in the NPT ensemble, with a timestep of 1 fs, with the Nose-Hoover thermostat : 2 ns at 300 K followed by a rise in temperature to 800 K over 4 ns. After that, the temperature was maintained at 800 K for 5ns, then decreased to 300 K in 4 ns, before being maintained during 25 ns at 300 K. For the FEP calculations 41 steps with 50 ps of equilibration and 100 ps of acquisition were performed. These FEP and MSD calculations were performed using the LAMMPS package [57].

3. Results and discussion

3.1. Adsorption of insulin aspart hexamer

Insulin (and two of its excipients, phenol and metacresol) were quantified after static contact with the different PVC tubes. Fig. 1a) represents the change in concentration (expressed as a % of the initial concentration) of insulin aspart as a function of time. Insulin concentrations decreased even after only 15 minutes of contact to an average (and for all the tested types of tubings) comprised between 95% and 90% of the initial concentration during the first 6h period (and between 90% and 85% after 24h contact). The increased variability in the insulin quantification results during the contact study can be attributed to small variations in the insulin-tubing interactions. This data is in adequation with previously published observations concerning the interaction of insulin aspart with various polymer surfaces. Interestingly, the presence of plasticizers (and their chemical nature) did not influence the loss of insulin aspart, which maxed out very quickly. This phenomenon will be discussed more in-depth further on in the discussion.

To investigate this more in detail, PMF calculations were carried out. Despite the difficulties which are inherent to the application of this methodology to large molecules like the insulin aspart hexamer, Fig. 2 presents the results of these intensive calculations. Indeed, Fig. 2 shows the Gibbs free energy profile along the separation distance between the center of mass

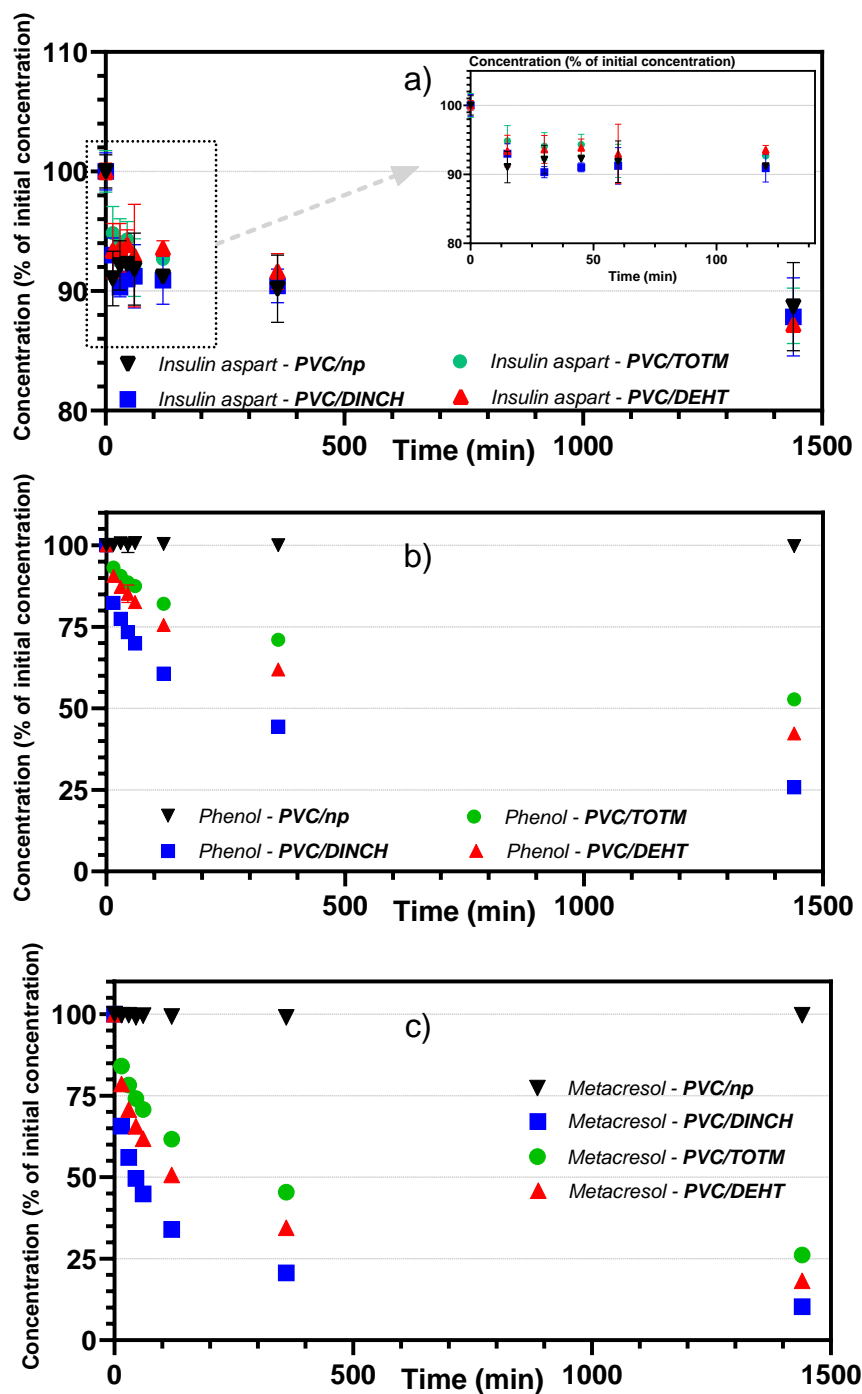


Figure 1: Evolution of insulin (a), phenol (b) and metacresol (c) concentrations (in% of the initial concentration) over 24 hours in static contact with non-plasticized (PVC/np) and plasticized polyvinyl chloride tubings (n= 3, results given as mean and 95% confidence interval). Some error bars aren't visible (for 1b and 1c) because they are smaller than the symbols.

of insulin and the material in the direction normal to the surface. During these simulations, only the separation distance along the z -direction between the PVC surface and insulin was restricted through the use of a harmonic constraint. It means that the conformation and the rotational position of the insulin

hexamer was free to change during the simulation if it was energetically favourable. Interestingly, all the profiles show a negative free energy minimum indicating that the adsorption of insulin onto PVC surfaces is favorable from a thermodynamic viewpoint whatever the chemical nature of the surface

(PVC polymers or plasticizers). The free energy of adsorption which can be assimilated to the free energy minimum ranged from -142 kJ mol^{-1} to -25 kJ mol^{-1} as a function of the nature of the PVC material. More specifically, we observed that the free energy of adsorption was much more favourable for a non-plasticized PVC surface than for a plasticized PVC surface. At first sight, this seems to contradict the experimental results which show that the loss of insulin in Fig. 1a is independent of the nature of the PVC surface, whether plasticized or not. This result led us to perform a deeper analysis from both structural and energy point of views

Fig. 3a shows a snapshot of the insulin hexamer interacting with a PVC/DINCH surface after 200 ns of molecular dynamics simulations. It can be observed that the complex hexameric structure interacts with the surface with a configuration allowing 3 monomers to be in contact with the surface, thus orientating the Zn^{2+} cations in a top-bottom way. As it can be seen, this snapshot suggests that the PVC surface is mainly populated by plasticizer molecules, but of course it cannot (on its own) be taken on face value, which is why this observation was checked through the calculation of relevant properties.

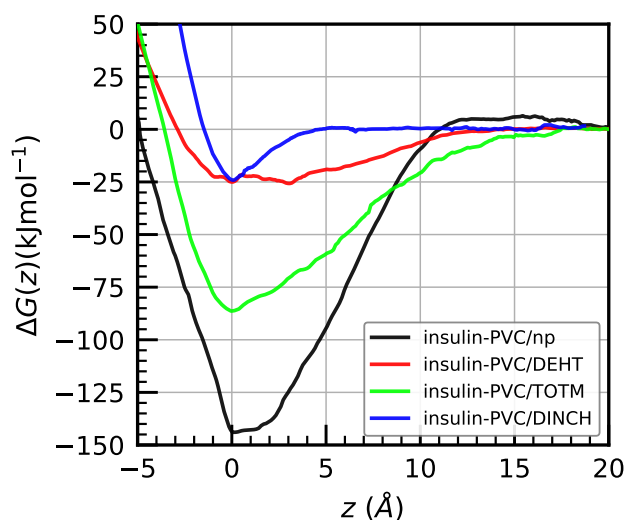


Figure 2: Gibbs free energy profiles (kJ mol^{-1}) calculated during the adsorption process of insulin aspart hexamer on non-plasticized (PVC/np) and plasticized polyvinyl chloride surfaces, calculated along the z -coordinate normal to the surface of the material.

Indeed, in order to propose an accurate description of the different molecular arrangements within the PVC material and at the PVC-water interface, we calculated the density profiles of PVC, insulin, water and plasticizers along the z -direction, which is the direction normal to the surface and along which the heterogeneity in density arises. Fig. 4a shows the density profiles of the insulin hexamer interacting with a non-plasticized PVC material. These profiles, calculated along the z -direction, indicate that the adsorbed insulin covers a region of approximately 45 \AA long in the direction normal to the surface. Over this region, we observe a strong depletion of water molecules in the density profiles of water due to the large space occupied by

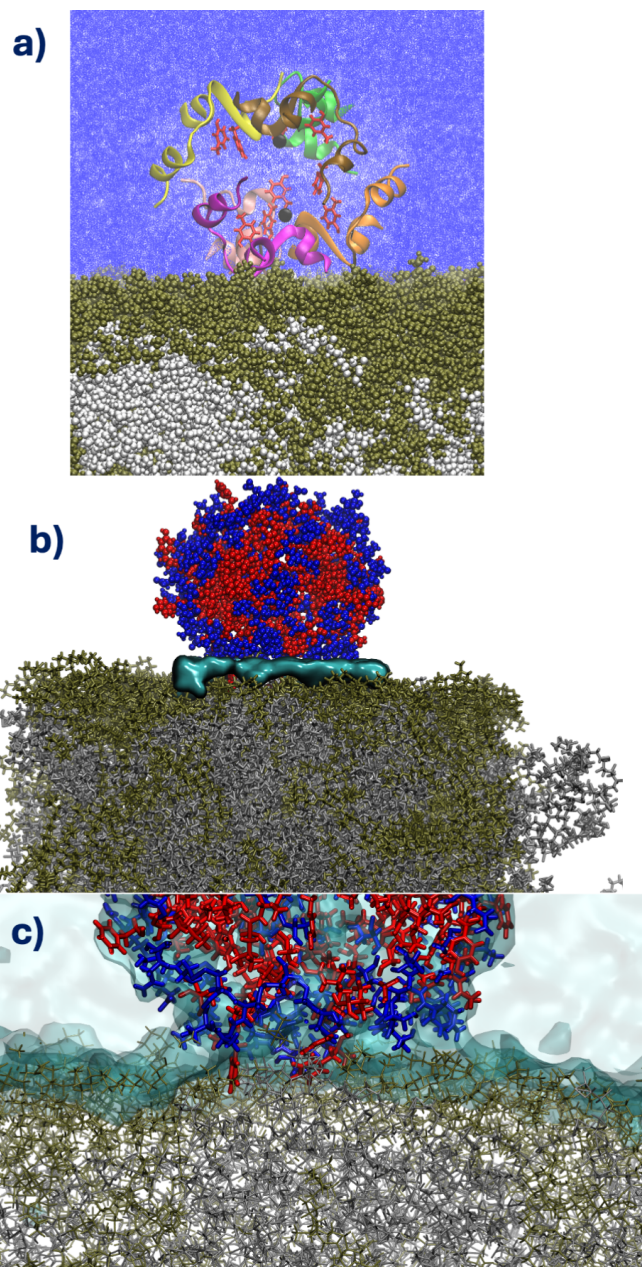


Figure 3: a) Snapshot of insulin aspart hexamer adsorbed to a PVC/DINCH surface (PVC: silver spheres, DINCH: tan spheres), including compounds stabilizing the quaternary structure: metacresol (red) and Zn^{2+} cations (the 2 dark grey spheres); b) Interfacial region, showing the protein in contact with a layer of water and plasticizer molecules concentrated at the surface of the polymer slab; and c) Protein adsorption is predominantly driven by hydrophilic amino acid residues (shown in blue licorice representation), in contrast to hydrophobic residues (shown in red licorice).

the adsorbed insulin hexamer. We also observe that at the interface the changes in density are abrupt, thus indicating no penetration of water molecules in the non-plasticized PVC material. Fig. 4b shows the area occupied by the DINCH molecules

in plasticized PVC, but is also indicative of an increased presence of plasticizer molecules at the solid-liquid interface, in direct contact with insulin and water molecules. These density profiles therefore confirm the presence of plasticizer molecules within the polymer matrix but also and most importantly they establish the formation of a layer of plasticizers at the surface of PVC, in interaction with water and insulin molecules. Fig. 3b illustrates this interfacial region, showing the protein in contact with a layer of water and plasticizer molecules concentrated at the surface of the polymer slab. The interface thus formed is much less well defined than in Fig. 4a. This layer of DINCH molecules can be viewed in Fig. 3 as the tan spheres representing the plasticizer on top of the surface. However, Fig. 4b does not allow us to conclude whether a layer of plasticizers is located between the insulin and the PVC surface or whether plasticizer molecules are located on either side of the insulin at the same z -point. In order to have a more detailed view of the adsorbed region, we focused on the xy region that is located around the center of mass of the insulin hexamer, by restricting the calculation of the density profiles to atoms that are located inside a z -oriented cylinder with a radius of 11 \AA which is the xy component of the insulin radius of gyration. The density profiles for each species are shown in Fig. 4c. They are shifted for each system in order to match the same location for the first peak of insulin density. These density profiles clearly establish a layer of plasticizer molecules interacting with both insulin and water molecules. The fact that the zone of interaction with insulin is mainly populated with plasticizers could be a key-element to interpret the simulated free energies of adsorption.

To quantify the impact of this layer of plasticizers on insulin adsorption, we report in Fig. 5 the number of amino acids which interact with PVC chains and with plasticizer molecules for the non-plasticized and plasticized PVC surfaces. In the case of non-plasticized PVC, 27 amino acids interact preferentially with PVC chains whereas for plasticized PVC materials, a number of amino acids ranging from 22 to 27 develop interactions (albeit of a weaker energetic level) with plasticizer molecules, as illustrated by the values of free energy of adsorption. This supports the conclusions drawn from the analysis of the density profiles *i.e.* that insulin interacts mainly with plasticizer molecules as it adsorbs on plasticized PVC surfaces. As illustrated in Fig. 3c, most of the amino acid residues in contact with the plasticized PVC are hydrophilic.

Additionally, theoretical [58, 59] and experimental [60] work have previously reported phase separations between plasticizers and polymer chains in some plasticizer materials. It can be deduced that at the molecular level some separated regions of plasticizers and polymers coexist. The segregation into distinct phases results from a balance between PVC-plasticizers interactions and self-associations. We have already shown the driving role of molecular interactions between the plasticizer and solvent in the migration of plasticizers from the PVC matrix [43]. The micro-scale phase separation between plasticizers and polymers is also a process that can be the origin of the migration of plasticizers within the material. Whereas the free energy of adsorption is significantly more negative when insulin

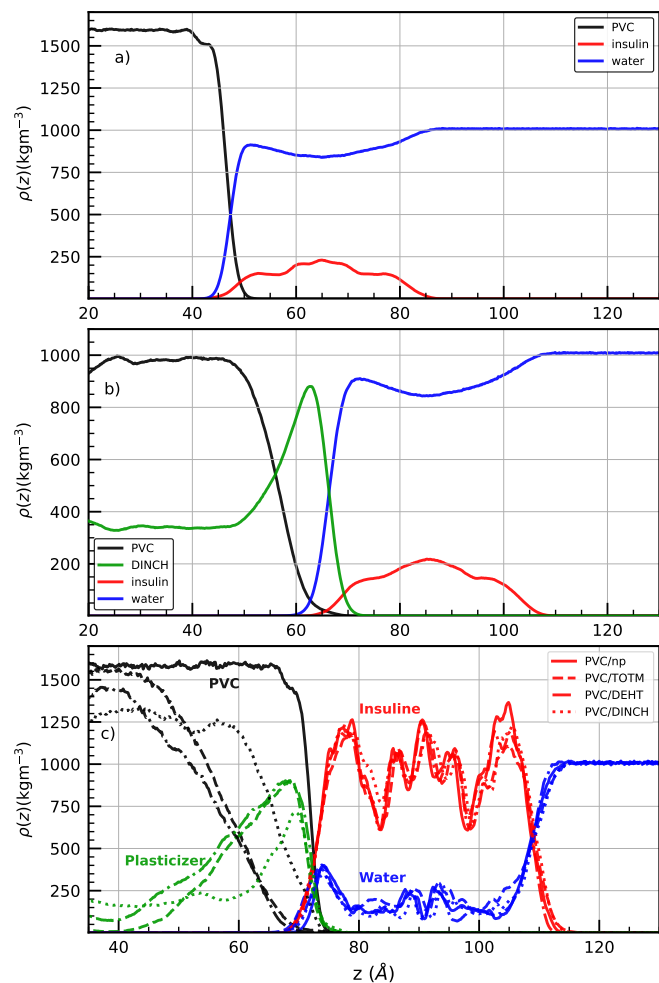


Figure 4: Density profiles of PVC, plasticizer, water and insulin molecules for a) nonplasticized PVC and b) DINCH/PVC surfaces; c) density profiles of PVC, plasticizer, water and insulin molecules calculated within a cylinder of a radius of 15 \AA along the z -direction normal to the PVC surface. These density profiles were averaged over 200 ns molecular dynamics simulations.

adsorbs on PVC chains, the adsorption of insulin onto interfacial regions formed by plasticizers is found to be favorable but to a lesser degree. The fact that the loss by sorption measured by experiments does not change with respect to the nature of the PVC, would mean that the insulin would adsorb mainly and firstly on polymer-rich regions in the plasticized PVC materials but that surface adsorption onto plasticized regions is still possible and energetically favourable, even if its contribution to the total free energy is much lower. This is the key-result of these potential of mean force calculations.

Interestingly, whereas atomistic simulations are able to capture the intricate molecular interactions driving the adsorption process, they are not yet able to model materials large enough to observe full-scale phase separations between polymers and plasticizers. This limitation is not due to the model or the method, but rather to the computational effort required to simu-

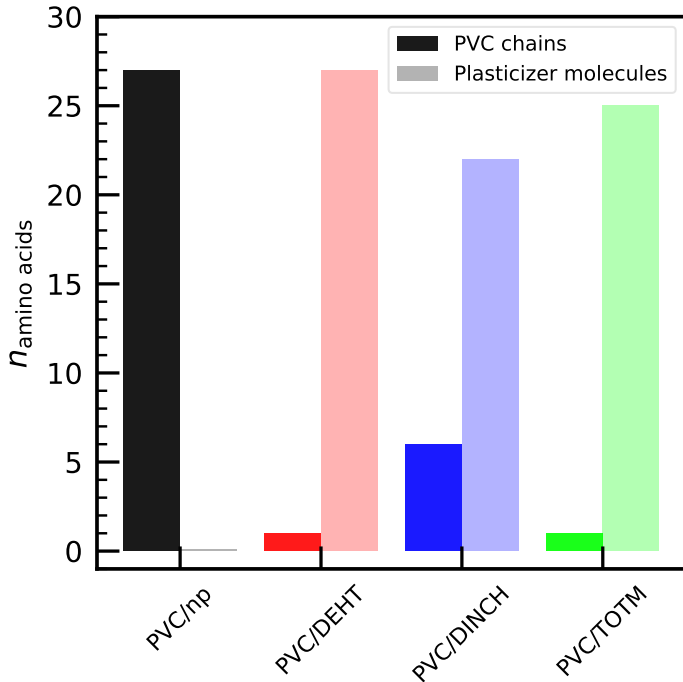


Figure 5: Number of amino acid molecules in interaction with the polymer chains of the PVC material and with the plasticizer molecules of the material calculated for each system. An amino acid is considered as being in contact with the surface if one of its atoms is at a distance smaller than 3.5\AA from a surface atom.

late such large system-sizes. One solution would be to simplify the model by using a lower resolution than that obtained when using atomistic force fields. The resulting coarse-grain simulations can then simulate larger polymeric systems and investigate phenomena such as phase separation that relax over longer time and larger length scales. Such coarse-grain models have been used to investigate the mechanism of plasticizer exudation from PVC material [59]. We could use such models to study the adsorption of insulin onto plasticized surfaces with distinct phases of plasticizers and polymers. However, this remains a project outside the scope of this paper but the PMF curves calculated here would be useful for checking the validity of the coarse-grain models [61].

The charge profiles shown in Figs. 6a and 6b confirm the electroneutrality within the polymer material and in aqueous solution far from the interfacial region. However, for the non plasticized PVC material, we observe in Fig. 6a that intense sequential layers of opposite charges (composed of PVC and water) organise themselves at the liquid-solid interface whereas the insulin hexamer contributes to this region mainly by negative charges, partly balanced by positive charges of water molecules. For the DINCH-PVC surface, Fig. 6b establishes that the presence of plasticizers destructures or attenuates these ionic layers by showing flatter charge profiles. By making the PVC material smoother, the plasticizer DINCH molecules provide a better balance of electrostatic charges at the surface of the material.

Interestingly, surface Zeta potential measurements displayed in Fig. 6c show that the intensity of the negative Zeta potential is weakened for plasticized PVC compared to non-plasticized PVC, quite likely because of the presence of the apolar plasticizers molecules at the surface. These experimental measurements are coherent with the calculations of the profiles of charges for plasticized DINCH and support the conclusions drawn from them that the distribution of charges at the surface of the plasticized PVC presents a more homogeneous distribution, with less alternating and less intense electrochemical positive and negative layers. However, despite these modifications, overall the solid surface remains profoundly electronegative (acidic), with surface Zeta potential values of around -60 mV , thus indicating that despite the thin layer of plasticizers forming itself at the solid-liquid interface, this isn't enough to fundamentally change the surface's capability to attract solvated insulin as shown by negative free energy of adsorption in Fig. 2.

We now return to the molecular description of the adsorbed insulin. Fig. 4c shows that some water molecules remain inside the hexamer, as documented in other studies[16, 29]. Indeed, this profile confirms the presence of a cavity inside the hexamer which can accommodate an average of about fifteen water molecules in line with the number calculated in a previous work [16]. The general shape and orientation of the insulin hexamer can be characterized by the radius of gyration tensor. The insulin in water is used as a reference system. All R_G values as well as the eigenvalues of the radius of gyration tensor are listed in Table 1. The two main eigenvalues, averaged over the different systems, $\lambda_1 \approx 1.18\text{ nm}$ and $\lambda_2 \approx 1.16\text{ nm}$ are almost the same and slightly greater than the third one $\lambda_3 \approx 0.94\text{ nm}$. This is typical of an almost spherical oblate shape. Similar sets of values are observed for the 123 components in each adsorbed system, showing that the eigenaxis remain correlated to the surface frame. Additionally, the angle between the main eigenaxis and the z axis normal to the surface is fairly constant, around 90° and no noticeable difference can be observed between the different systems. Therefore, no major change is observed in the general shape of the insulin hexamer as it is adsorbed onto the different surfaces. The shape and orientation are stable throughout the simulation as indicated by the very small fluctuations in all the R_G components. The insulin and water profiles show a very stable structure throughout the time of the simulation, indicating that the insulin molecule and its inner water molecules stays very still during the simulation. Additionally, the comparison between the different systems shows a strong similarity between the insulin and the water profiles. We conclude that the nature of the surface does not impact the structure of the adsorbed hexamer. The number of hydrogen bonds (n_{HB}) between insulin and water molecules ranges from 516 to 537 for all the systems (see Table 1) whereas the number of hydrogen bonds between insulin and the plasticized PVC surfaces is insignificant and less than 2 as shown in Table S1 of the Supplementary material. Interestingly, no decrease in n_{HB} was observed in the case of adsorbed insulin hexamer indicating that the hydrogen bond network of the insulin is not impacted by the surface. Furthermore, the analysis of the sec-

Table 1: Eigenvalues λ_1 , λ_2 and λ_3 of the radius of gyration tensor (R_G) calculated along the acquisition phase, for each system. All values are in nm. The fluctuations over time were less than 0.1 nm in all cases. n_{HB} is the number of hydrogen bonds between insulin and water molecules. The criteria for selecting the hydrogen bond was given in ref.62. n_p , n_s and n_- are the mean numbers of amino-acid in the kappa-helix, bend and loop secondary structures.

System	λ_1	λ_2	λ_3	R_G	n_{HB}	n_p	n_s	n_-
WATER	1.19	1.15	0.93	1.90	516	8.0	202.4	95.6
PVC/np	1.19	1.15	0.94	1.90	518	6.5	203.1	96.4
PVC/TOTM	1.19	1.17	0.94	1.92	537	7.1	202.7	96.2
PVC/DEHT	1.19	1.17	0.93	1.91	530	5.4	203.9	96.6
PVC/DINCH	1.17	1.14	0.95	1.90	517	4.9	203.4	97.7

ondary structure via the DSSP module in GROMACS and the n_p , n_s and n_- numbers revealed that the adsorption of insulin onto the PVC slab (plasticized and non-plasticized) did not lead to notable structural alterations (Table 1). This observation, together with the consistent radius of gyration and the stable number of hydrogen bonds, confirms that insulin maintains its structural integrity (both secondary and tertiary structures) during adsorption onto the polymer surfaces. This information is quite important as protein adsorption to hydrophobic surfaces can lead to protein denaturation, our results add weight to the hypothesis that this is probably not the case with hexameric insulin apart.

3.2. Absorption of phenolic excipients

Figs. 1b and 1c present the results of the quantification of phenol and metacresol, respectively, during 24h of contact between the solution and the polymer tubes. The mean concentrations of these compounds remained unchanged (maximum 0.25% and 1.02% loss for respectively phenol and metacresol) during the first 24h when in contact with non-plasticized PVC, but reaching 3.1% and 3.5% after 14 days of contact (see Fig. S1 of the Supplementary material). However, the concentrations decreased gradually over time (fastly at first, then with slower kinetics) when in contact with plasticized PVC for up to 24h, to reach losses comprised between 47% and 74% for phenol and 74% and 90% for metacresol. As adsorption is generally characterized by a fast interaction followed by a plateau reached in a couple of minutes [63], and absorption as a much slower process that follows adsorption [64], it is reasonable to estimate that phenol and metacresol diffused (absorbed) into the plasticized PVC bulk (but not into the non plasticized PVC). It can also be noticed that metacresol losses were about 50% higher than phenol losses, and that for both molecules the losses were increased depending on the type of plasticizer used: TOTM < DEHT < DINCH.

These results are consistent with previously published data which also found higher losses for phenol and metacresol than for insulin during a 24h infusion through DINCH plasticized PVC tubings [37]. Losses of metacresol higher than those of phenol have also been previously reported when solutions containing these compounds were put into static contact with silicone tubes [36, 42].

The most obvious chemical reason explaining the difference in losses between these very similar compounds would be the

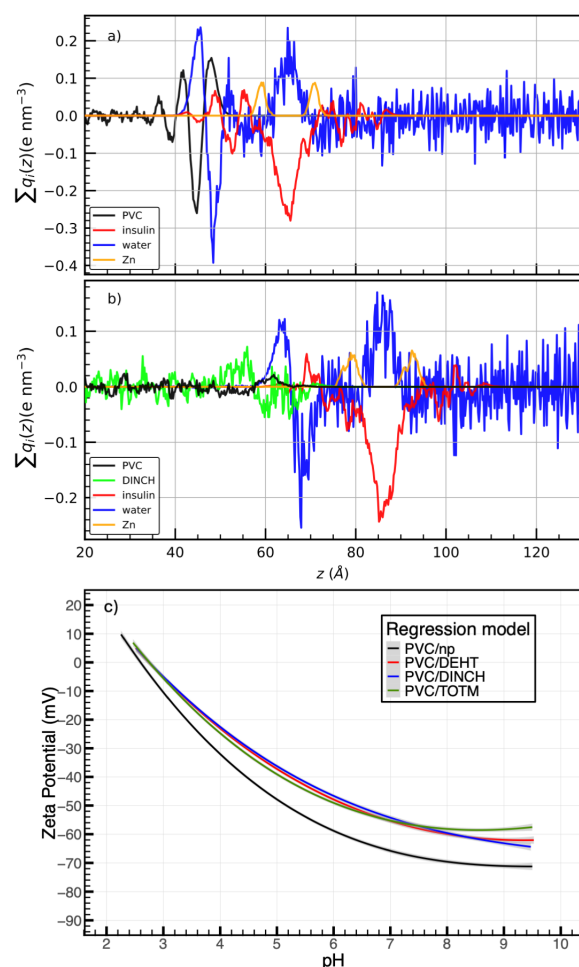


Figure 6: Charge density profiles of a) nonplasticized PVC and b) plasticized DINCH/PVC surfaces; c) evolution of surface Zeta potential as function of pH for surfaces of non plasticized PVC and PVC plasticized with DEHT, DINCH and TOTM.

extra methyl group of the metacresol structure, conferring it an increased lipophilicity (logP of 1.96 compared to 1.46 for phenol [65]) and thus an increased affinity for the PVC chains

which would gain an increased mobility/permeability from the highly lipophilic plasticizers, in stark contrast with the non-plasticized PVC which did not cause any excipient loss. This study also confirms that the nature of the plasticizer used in plasticized PVC can influence the loss of chemical compounds, in this case pharmaceutical excipients. Indeed, Al Salloum *et al.* did find that for laboratory-made PVC films containing 40% of different plasticizers (DEHP, DEHT, TOTM, DINCH) the nature of the plasticizer did have a small impact on the drug loss % of diazepam, with PVC/DINCH inducing slightly more sorption than the other plasticized PVCs [66]. In an older study, Treleano *et al.* also found differences in the sorption of two lipophilic APIs (nitroglycerin and diazepam) onto plasticized PVC tubes, but as the tubes had been manufactured to possess the same Shore hardness (80 Shore A in their case) the plasticizer content ranged from 23.4% (for PVC/DEHA) to 36.3% (for PVC/TEHTM = TOTM), inter-tube comparison cannot be made [67]. Sahnoune *et al.* showed that over the course of several days the drug loss of a lipophilic API (diazepam) was independent of the plasticizer used [68], but as the first analysis time point was after 24h contact and that only about 2% of diazepam was left in the solution, it is possible that the actual design of the study made it impossible to identify any potential differences. Plasticized PVC is a material well known for being the source of interactions with lipophilic compounds, so these results could open new interesting possibilities as it might be possible to devise and then manufacture a PVC with a plasticizer that doesn't promote sorption.

For the sake of exhaustivity, it must also be mentioned that during the analyses, an in-depth examination of the chromatographic data allowed the detection of the peaks of two unidentified compounds, possessing a retention time of 3.20 min (from solutions having been in contact with all three PVC plasticized tubings) and 3.75 min (only present in solutions having been in contact with DEHT plasticized PVC tubes). The area under curve of these peaks increased over time (see Fig. S2 in the Supplementary material). As this finding was beyond the scope of this manuscript, it will not be discussed further on.

The adsorption process must be decoupled from the absorption process. Molecular simulations make it possible to separate these two processes using appropriate methodologies. Since adsorption occurs before absorption, we investigated the ability of the metacresol and phenol molecules to adsorb on the surface of plasticized and non-plasticized PVCs by calculating the Gibbs free energy profile along their separation distance in the z direction perpendicular to the surface. For each excipient, 5 independent calculations were carried out. Fig.S3 of the Supporting Information shows the average free energy profiles with the statistical fluctuations represented with shaded zones. All these curves show negative free energy minima indicating that the adsorption is thermodynamically favored. The average free energy minima, considered in a first approximation as the free energy of adsorption, range from -25 to -50 kJ mol^{-1} and support the hypothesis of a physical adsorption governed by van der Waals interactions. First, in the case of plasticized PVC surfaces, experiments and molecular simulations are consistent with each other, *i.e.*, the loss by sorption of these excipients is

accompanied by a favorable free energy of adsorption. Second, in the case of the non-plasticized PVC, whereas experiments show no loss by sorption of the excipients over 1500 min (see Figs. 1b and 1c but a 3% loss after 14 days as shown in Figs.S1a and S1b of the Supplementary Material), molecular simulations would tend to show that the adsorption is possible from a thermodynamic viewpoint. The analysis of the PMF curves on their own does not allow us to elaborate further on the absorption process because the simulation time is not long enough to allow the plasticized PVC material to relax and the excipient to penetrate and diffuse into the material. We can even assume that this time would be much longer for non-plasticized PVC, as the absence of plasticizers would lead to a slower relaxation of the material.

To investigate the absorption process, we proposed an alchemical transformation consisting of progressively canceling the interactions between the encapsulated excipient molecule and the PVC material. This calculation will give us information about the spontaneous nature of the transformation, without having to consider its kinetics. We calculated the difference of the Gibbs free energy at different stages characterized by the coupling parameter λ by using different methodologies [33]. The reader is redirected to the Supplementary material for a comparison between the FEP, TI and FDTI methodologies [33] and a comprehensive description of the operational expressions (Eqs. S3-S9). The initial state defined by $\lambda = 1$ corresponds to a system where the adsorbed excipient and the polymer matrix fully interact whereas $\lambda = 0$ defines a system in which the excipient and PVC material no longer interact. If we apply the same methodology for an excipient molecule in water by switching off gradually their intermolecular interactions, we obtain at the end of the transformation the opposite of the free energy of hydration. Fig. S4 shows the values of the free energy as a function of the coupling parameter λ during the cancellation of the interactions between the metacresol molecules and its environment represented here by either water molecules or atoms of the polymer matrix. From these free energy contributions, it is possible to calculate the free energy of hydration of these excipients as well as the free energy of absorption (see Eq. S13 of the Supplementary material for the different relationships). The values of these free energies are given in Table 2 for the metacresol and phenol molecules. The values of the free energy corresponding to the annihilation of intermolecular interactions between excipient molecules and PVC materials are presented in Table S2 of the Supplementary material. Fig. S4 shows that the free energy values of $\Delta G_{\text{water-void}}$ and $\Delta G_{\text{polymer-void}}$ related to the annihilation of the interactions between the excipient molecule and the water molecules on the one hand and between the excipient and the polymer matrix on the other is positive, thus indicating that the reverse transformations of hydration and absorption from vacuum are thermodynamically favorable, respectively.

The free energy of absorption of metacresol and phenol molecules into plasticized PVC materials (see Table 2 and Fig. 7) are negative, thus indicating that absorption would be a spontaneous phenomenon. These negative values also indicate that the favorable interactions between the excipients and

Table 2: Gibbs free energy of hydration of metacresol and phenol molecules along with free energy of absorption of non-plasticized and plasticized PVC materials. We also give for comparison the free energy of absorption of the excipient molecules into a bulk of plasticizers. The standard deviations were calculated over 5 independent calculations.

Medium	metacresol	phenol
$\Delta G_{\text{hydration}} \text{ (kJ mol}^{-1}\text{)}$		
Water	-16.6 ± 0.5	-17.2 ± 0.4
$\Delta G_{\text{absorption}} \text{ (kJ mol}^{-1}\text{)}$		
PVC/np	-18.1 ± 4.9	-10.4 ± 4.0
PVC/DEHT	-19.1 ± 8.7	-17.8 ± 3.2
PVC/DINCH	-21.6 ± 6.4	-17.6 ± 2.9
PVC/TOTM	-21.3 ± 5.4	-12.2 ± 3.6

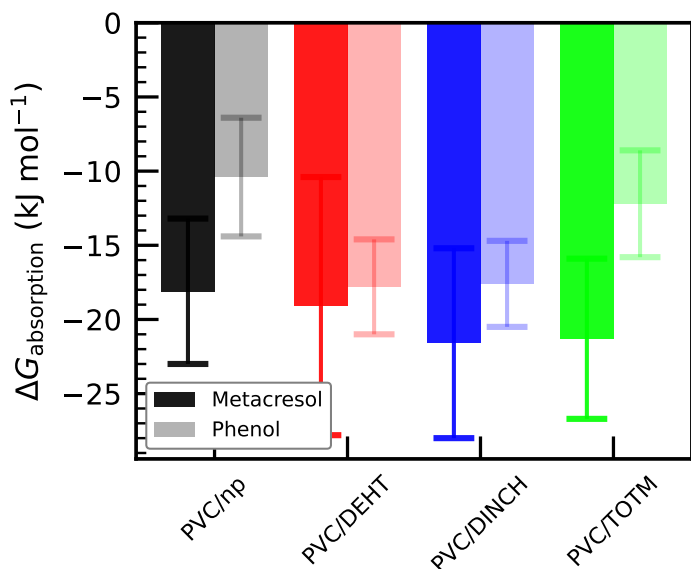


Figure 7: Absorption Gibbs free energy values calculated for metacresol and phenol excipients molecules in nonplasticized and plasticized PVC surfaces .

the plasticized polymer matrix make the absorption possible, in line with the interpretation of losses of excipients measured experimentally. This is a key conclusion that could only be reached thanks to a combination of experiments and specific molecular simulation algorithms. In addition, the FEP calculations shown in Fig. 7 establish that the free energy of absorption of metacresol is more favorable than that of phenol suggesting that metacresol losses would be greater than for phenol. This conclusion is supported by the experimental results reported in Figs. 1a and 1b, therefore bringing added weight to the validity of the simulations and the conclusions that can be derived from it.

Concerning the non-plasticized PVC material, interpreting the results is trickier. Indeed, the free energy of absorption remains negative but slightly less favorable than for plasticized PVC surfaces. These contributions are subject to strong variations due to the heterogeneity, slower diffusion of encapsulated excipients and insufficient relaxation of the PVC material. For this PVC material that relaxes over longer times, we would need to improve the overall methodology for calculating the free energy of absorption and to extent significantly the number and length of the simulations in order to obtain precise data, but developing such methods would be out of scope of the present work and may require the use of coarse-grained models. Therefore, to investigate this point further with the methodology and resources currently available to researchers, we turned our attention to the mobility of the phenolic excipients within the PVC matrix. To do so, we calculated the mean squared displacement (MSD) as a function of time. The calculated MSDs are shown in Fig. S5 of the Supplementary material for phenol and metacresol excipients within the matrix of a non-plasticized PVC and a plasticized PVC/DINCH. Firstly, we can observe that the mobility of the phenolic excipients phenol and metacresol is increased in plasticized PVC compared to non-plasticized PVC. These results support the idea of a possible mobility and therefore diffusion process of these small molecules into the bulk of plasticized polymers at the molecular scale. This also is perfectly in line with the experimental data reported by Saller *et al.* who proved experimentally using a diffusion Franz cell that phenol and metacresol diffused completely through a polymer (silicone) membrane of 2.1 mm after respectively 40 and 56 minutes [36]. Indeed, to arrive into the acceptor cell, the molecules had to first adsorb onto the surface, then diffuse through the tubing wall, then be released into the acceptor cell media. Interestingly and from a simulation point of view, our work also confirms the challenge of sampling properly the non-plasticized PVC material and then of calculating a reliable free energy of absorption. These mean square displacements which can be related to the diffusion coefficient allow the rationalization of the experimental drug loss profiles, during which phenol and metacresol concentrations continue to decrease over time whereas the insulin concentrations decreased sharply at the beginning before quickly (in less than 15 minutes) stabilizing.

The negative sign of free energy of absorption shows that the partition coefficient of the excipients between water and the polymer tube is in favor of the latter. This means that absorp-

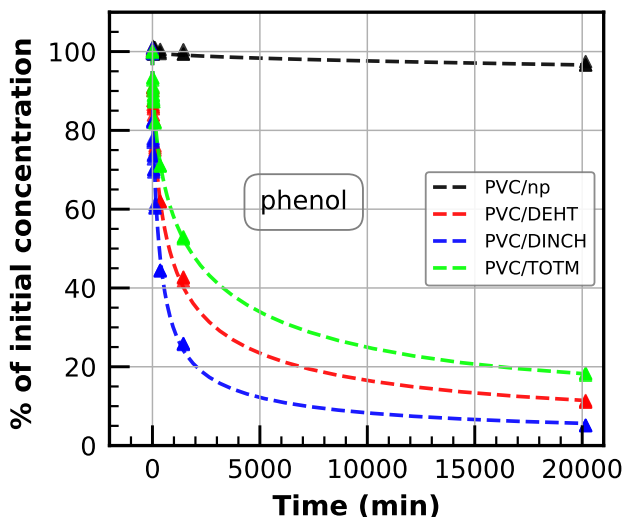


Figure 8: Phenol remaining in solution as a function of time for tubings made of PVC with different plasticizers. The symbols are experimental measurements whereas the dotted lines are the results of fits obtained with the properties derived from simulations. The same curve for metacresol is shown in Fig. S7 in the Supporting information.

tion is expected to play a major role in the interaction process of the excipients with the polymer surface. This is in accordance with other papers in the literature that studied the loss of preservatives when in contact with polymer materials [64]. Consequently, we developed a macroscopic diffusion model taking into account: 1) the diffusion inside the water media and inside the tube wall; 2) the partition coefficient fixing the equilibrium ratio of concentration; and 3) the adsorption at the interface. This is a first step toward quantitative prediction of the loss by sorption. More details about the model are given in the Supplementary material. By using the adsorption length calculated from the PMF curves, the diffusion coefficients obtained from MSDs, the partition coefficient deduced from $\Delta G_{\text{absorption}}$ and the experimental geometry of the tubings, it is possible to simulate the evolution of the concentration profiles, and the loss of excipients as a function of time. Very interestingly, Fig. 8 shows that the diffusion model is able to reproduce the experimental data obtained for phenol. The values of the fitting parameters, given in Fig. S6 of the Supplementary material, are within the error bars of molecular simulations, excepted for the free energy of absorption of excipients in a non-plasticized PVC. The diffusion model also shows that the free energy of absorption need to be better predicted by molecular simulations. The fitting curve of the evolution of concentration of metacresol as a function of time is given in Fig. S7 of the Supplementary material. This diffusion model shows that the main cause of phenol and metacresol sorption during long experimental times is adsorption, and not the adsorption at the interface, excepted perhaps with non-plasticized PVC.

Interestingly, it is often observed in biased molecular simulations using PMFs that the local $\Delta G(z)$ value increases and becomes positive (see Fig. S3) as the molecules approaches the

surface of the polymer material. This may be an unexpected result due to the limited simulation time which is significantly shorter than the polymer relaxation time and prevents an exhaustive sampling of the material. But it does not mean that the absorption is not possible or unfavorable at the macroscopic time scale. This same time limitation may explain the overestimation of $\Delta G_{\text{absorption}}$ (in absolute value) because the polymer doesn't have enough time to fully rearrange itself during the cancellation of the interactions with the excipient.

4. Conclusion

We have conducted a study combining molecular simulations and experiments in order to obtain a thermodynamic characterisation of the interactions occurring between the insulin aspart hexamer and its excipients with plasticized PVC surfaces. In a first stage, the experimental data showed that the loss profiles of insulin hexamer on the different PVC surfaces that were consistent with an adsorption of this biomacromolecule. The experiments also showed that the loss of insulin by sorption was independent of the nature of the PVC surface. Molecular simulations and more precisely calculations of the Gibbs free energy profiles along the separation distance established that the interaction between the insulin hexamer and the PVC material is thermodynamically favorable. More specifically, the molecular simulations revealed that the insulin molecule can adsorb onto a layer of plasticizers at the surface of the PVC even if this adsorption is much less favorable from a free energy viewpoint than the adsorption on PVC-rich regions. Reconciling experiments and free energy calculations required considering the formation of distinct polymer and plasticizer phases within the plasticized PVC. This implies that insulin adsorbs preferentially on PVC-rich regions in plasticized PVC. Investigating this interpretation further by simulations would require the development of coarse-grain simulations in order to simulate PVC materials with different phases of polymers and plasticizers and to evaluate the adsorption of insulin on these different regions. However, the atomistic simulations carried out here on the adsorption of insulin aspart are relatively rare and will be very useful for future coarse-grained simulations which will need validation of the energetic characterisation of the adsorption. We completed the study by a comparison of the properties of the insulin hexamer upon adsorption to those in bulk water solution. The methodology we used in this study could be expanded to other biomacromolecules and other situations, such as for example protein adsorption onto implantable biomaterials or bio-fouling.

In the case of the phenolic excipients such as metacresol and phenol molecules which are critical for maintaining insulin aspart stability and sterility, the aspect of the excipient loss curve over time and the high level of loss both support an adsorption of the excipient into the PVC material. Because standard molecular simulations are not capable of simulating long enough times to observe the penetration of the excipient and its diffusion within the material, we applied biased methodologies that calculate the absorption free energy without considering the kinetics. Our simulations confirmed that the free energy

values of absorption are favorable for plasticized PVC surfaces in line with the losses observed from experiments. We used the properties calculated from molecular simulations, *i.e* the diffusion coefficient of the excipients into the plasticized PVC, the free energy of absorption of the excipients and the adsorption length calculated from the free energy curves of adsorption to develop a macroscopic diffusion model. Very interestingly, we demonstrated here that the diffusion model performs very well in the reproduction of losses of excipients as a function of time for the plasticized PVC material. Achieving a better agreement with experiments in non-plasticized PVC surfaces would require the development of a methodology devoted to the calculation of the free energy of absorption for systems which relax over very long times, inaccessible to molecular simulations. Using coarse-grained models and mesoscopic simulations could be an alternative.

The combination of theory, advanced molecular simulation methods and experiments gives considerable added-value to the study of adsorption of biomacromolecules. This interdisciplinary approach not only facilitates a deeper understanding of molecular-scale adsorption and absorption processes but also enables the use of simulated properties to reproduce the sorption behavior of these biomacromolecules on a macroscopic scale.

Credit author statement

Pierre Fayon: Investigation, Conceptualization, Writing-Original Draft preparation, **Philip Chennell:** Investigation, Conceptualization, Supervision, Validation, Writing-Original Draft preparation, Writing - Reviewing and Editing **Mehdi Sahihi:** Conceptualization, Investigation, Writing - Reviewing and Editing **Julien Devémy:** Methodology, resources, software **Emmanuelle Feschet:** Methodology, Investigation, Writing - Reviewing and Editing **Lionel Nauton:** Methodology, Investigation, Writing-Original Draft preparation **Florent Goujon:** Conceptualization, Investigation, Writing - Reviewing and Editing **Alain Dequidt:** Supervision, Conceptualization, Investigation, Writing - Reviewing and Editing **Valerie Sautou:** Supervision, Conceptualization, Project administration, Writing - Reviewing and Editing **Patrice Malfreyt:** Conceptualization, Supervision, Validation, Writing-Original Draft preparation, Writing - Reviewing and Editing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Some input, parameters and force field files used in GROMACS can be downloaded at this address : <https://git.mesocentre.uca.fr/simatlab/insulin-interactions-pvc>. Supplementary data to this article can be found online at ...

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