Development of monoclonal antibodies in tablet form: A new approach for local delivery

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A B S T R A C T

Among the various pharmaceutical forms, tablets offer numerous advantages, like ease of administration, cost-effectiveness in production, and better stability of biomolecules. Beyond these benefits, the tablet form opens up possibilities for alternative routes for the local delivery of biopharmaceuticals such as oral or vaginal administration, thereby expanding the therapeutic applications of these biomolecules and overcoming the inconvenients associated with parenteral administration. However, to date there is limited information on the feasibility of developing biomolecules in the tablet form.

In this study, we have evaluated the feasibility of developing monoclonal antibodies in the tablet form while preserving their biological properties. Different excipients and process parameters were studied to assess their impact on the antibody's integrity during tableting. ELISA results show that applying compression pressure up to 100 MPa is not detrimental to the antibody's binding properties when formulated from a lyophilized powder containing trehalose or sucrose as the major excipient. This observation was confirmed with SPR and ultra-centrifugation experiments, which demonstrated that neither the binding affinity for both Fc and Fab antibody fragments nor its aggregation rate are affected by the tableting process. After compression, the tablets containing the antibodies have been shown to be stable for 6 months at room temperature.

1. Introduction

Since the introduction of the first therapeutic monoclonal antibody (mAb) in 1985 (Muromonab) (Ecker et al., 2015), mAbs have experienced significant growth in the biopharmaceutical industry. This growth is reflected in a few key statistics: 13 new antibodies (or derivatives) obtained a first approval in either the US or EU each year in 2018, 2021, and 2022 and 12 in 2020 (Kaplon et al., 2023). Furthermore, since the beginning of 2023, over 20 % of new drug approvals granted by the FDA's Center for Drug Evaluation and Research have been related to antibodies (Research, 2023). Throughout these years, the development of therapeutic mAbs has revolutionized the treatment of a broad range of diseases in various fields such as oncology, dermatology, neurology, autoimmune disorders or infectious diseases (Mullard, 2021). The great interest in therapeutic antibodies can be explained on the one hand by their multiple natural functions, including neutralization, opsonization, antibody-dependent cell-mediated cytotoxic (ADCC) activity, or complement-dependent cytotoxic (CDC) activity, which allow a wide range of pharmacological applications. On the other hand, therapeutic mAbs exhibits good safety profiles. This is partly due to their high antigenic specificity but also thanks to significant progress in their development methods, particularly through genetic engineering, which has progressively enabled the production of partially or fully humanized antibodies, resulting in reduced immunogenicity effects for clinical use in humans. Nonetheless, despite this attractive and promising market, the formulation of mAbs remains challenging. Antibody's characteristics such as poor intestinal permeability (high molecular weight) or proteinaceous nature that makes them sensitive to gastric pH and enzymes, quickly ruled out the possibility of administering them through the preferred non-invasive oral route and so, their development has so far been oriented on liquid or freeze-dried (FD) formulations for parenteral administration (intravenous, subcutaneous routes). Parenteral administration however, has several technical, clinical, or patient-related disadvantages. Firstly, the development of formulations associated

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with parenteral administrations requires the implementation of aseptic processes and, particularly for solution-based formulations, involves a logistical challenge associated to cold chain maintenance during their transport and storage. Secondly, parenteral administration results in poor patient compliance since administration generally requires a trained healthcare professional which can limit accessibility and increase healthcare costs. But ultimately, one of the major limitations is related to their pharmacokinetics properties. As large and polar molecules, antibodies have a low tissues distribution and are majorly confined within the vascular or lymphatic spaces following systemic administration. As a consequence, delivering antibodies to maintain the drug at therapeutic level is challenging and may require the administration of a large quantity of mAb or even multiply injections so that a sufficient quantity of molecules reaches the target organ/tissue.

Considering these limitations, the development of alternative dosage forms for local mAbs delivery may be highly revelant. This is particularly true for therapies based on passive topical immunization, where the absorption of the antibody is not necessary to obtain therapeutic activity. Examples can include the management of chronic conditions such as Crohn's disease or ulcerative colitis that currently rely on immunomodulation therapy with mAbs (infliximab, velodizumab), and for which local delivery of mAbs directly to the intestinal or rectal mucosas could be judicious to favor the local anti-inflammatory therapeutic effects and improve patient comfort. Other examples can also include common non-invasives bacterial, fungal, or viral infections of the buccal, gastrointestinal or vaginal mucosal surfaces. For these types of infections, a topical administration of antibodies has demonstrated effectiveness in preventing infectious agents from attaching and colonizing the epithelium (Lehner et al., 1985), or penetrating and replicating in the mucosa (Veazey et al., 2003; Yang et al., 2020). Such a therapeutic objective can be easily accessible by the use of conventional pharmaceutical forms for topical application.

Generally topical dosage forms consist of semi-solid preparations such as creams, gels, ointments, ovules or suppository. However, these forms typically contain liquid phases which can promote physicochemical instabilities of proteins during manufacturing and storage. Formulation of therapeutic antibodies into dry forms may therefore be highly preferable.

Among all available dosage forms, tablet is the most commonly used. This can be explained by its ease of administration, low production cost, stability and good patient compliance. Moreover, compared to other dosage forms, tablets offer numerous advantages such as precise dosing of the active substance, the ability to control and/or delay the release of the drug, and last but not least, tablet form can present an attractive opportunity for the local delivery of drugs, and more specifically mAbs, whether through buccal, gastrointestinal, rectal or vaginal mucosal administrations, with a time of exposure of mAbs to the mucosal surface that can be modulated.

Tablets are made by compressing a powder, which implies that the drug in solution form must undergo a prior drying process to attain a powdered state. For antibodies, the drying step is already well-established, with lyophilized powders making up 25 % of commercially available monoclonal antibody (mAb) formulations (Strickley and Lambert, 2021). Therefore, the freeze-drying process has been selected in this study as the method of choice to produce powder while preserving the bioactivity of the mAbs. In addition, previous works (Hsein et al., 2023; Madi et al., 2024) has demonstrated that freeze-dried powders present singular tableting properties specifically in terms of cohesion which reinforced our choice of freeze-drying to obtain the powder. However, the tableting process itself presents great challenges for the conservation of mABs activity. Actually, tableting process requires the application of high axial pressures resulting in mechanical stresses througout the compressed powder in order to generate cohesion between powder particles. The few studies carried out to examine protein compression have revealed that these stresses can potentially induce physical instabilities in proteins, leading to aggregation or conformational changes, which consequently have a detrimental effect on their biological activity (Klukkert et al., 2015; Wei et al., 2019).

Frijlink and his colleagues were the first to investigate the feasibility to develop mAb as a tablet pharmaceutical form (Gareb et al., 2019; Maurer et al., 2016). In these works, the tablets were manufactured from a FD powder obtained from the commercial product Remicade® (infliximab) within a glassy matrix of inulin. The primary objective of this study was not to explore the impact of the compression process on the antibody's integrity. Only informations concerning the dry granulation step via slugging have been disclosed but no information about the tableting process parameters were given. Nevertheless, results suggest that, within the specified operational parameters, the tableting process did not have any detrimental effects on the antibody's biological functions. No apparent alterations in the protein structure or loss in its binding capacity were observed compared to the original Remicade® formulation. The impact of tableting process on antibodies has also been recently studied (Lu et al., 2021). This study aimed to investigate the impact of various factors such as compaction pressure and particle size on antibodies integrity following compaction of a FD mixture of human polyclonal antibodies. Among the main results, this study revealed that high compression pressure (300 MPa) can lead to changes in the secondary structure of immunoglobulins. Furthermore, when studying the influence of compaction on the biological activity of these antibodies, it was demonstrated that their ability to bind to their antigenic target is adversely affected by compaction at both low and high compression pressures. Nonetheless, no correlation between pressure level and the impairment of antibody binding activity was identified.

Overall, these studies demonstrate the potential for developing therapeutic antibodies in tablet form. However, they suggest that both product and process parameters can negatively impact the integrity of antibodies. Nevertheless, their individual impacts have never been concurrently examined. Therefore, the aim of this study is to demonstrate the feasibility of manufacturing tablets using lyophilized powders incorporating monoclonal antibodies while preserving their physicochemical and biological properties for local administration. For this purpose, the critical parameters of the product-process pairs during the tablet production of mAbs were further investigated. To this end, multiple variable including excipients, mAb quantity and compression pressure were simultaneously explored in order to identify the key parameters that may ensure the preservation of mAbs biological activity when compressed into tablet form.

2. Materials and methods

2.1. Chemicals

Trehalose dihydrate (Goch, Germany) and sucrose (Goch, Germany) were generously given by DFE pharma. Polysorbate 20 (Calbiochem®), phosphate buffer saline (PBS) (Euromedex), magnesium stearate (Cooper), Tubulin (from porcine brain, Cytoskeleton), Protein G (Genscript) and secondary Anti-Mouse IgG (H + L) antibody (Jackson Immuno Research) were purchased from their respective suppliers.

2.2. TAT-1 mAb generation and purification

Experiments were carried out using mAb TAT-1 as a model antibody. We selected this antibody for our study because it belongs to the IgG class, the most common class of therapeutic antibodies. Additionally, we had access to the hybridoma cell line for this antibody ((EACC00020911) (Public England)), enabling us to manage its large-scale production effectively. TAT-1 cells were grown at 37 °C in an air-CO_2 5 % environnement in medium DMEM (Gibco®) supplemented with 10 % (v/v) heat inactivated fetal bovine serum (Eurobio Scientific) and antibiotics (penicillin/streptomycin, 50 μg/mL). Secreted antibodies were purified from culture supernatant by protein G column "HiTrapTM Protein G HP 1 mL" using AKTA purifierTM system (GE

Healthcare) according to supplier's recommendations. Following purification, sodium azide was added to the samples at a final concentration of 0,02 %, then samples were stored at 4 °C.

2.3. Freeze-drying

Freeze-drying was performed using a Cryotec® pilot freeze-dryer. Aqueous solutions of disaccharide (trehalose or sucrose) (5 % w/v) with polysorbate 20 (0.005 % w/v) and, in some cases, with mAbs (at a final concentration of 100 or 500 μg/mL) were prepared. The solution was then dispensed in 1 mL aliquots into vials before being loaded into the freeze-drying chamber. For the first step of the freeze-drying process, samples were frozen by cooling the shelves from 25 °C to −50 °C at a ramp rate of 1 °C/min with an intermediate stage held at −5°C for 20 min to ensure cooling uniformity across samples. At the end of the cooling step, the temperature was maintained at −50 °C for 60 min. Sublimation step was then performed by increasing the shelve's temperature from −50 °C to −20 °C at a ramp rate of 1 °C/min and at a pressure of 0.1 mbar. Conditions were maintained for 14 h. This duration was previously determined to ensure that the pressure values of capacitance and pirani manometers gauges came together. Finally, the secondary drying step were performed by raising shelve's temperature until 25 °C at a ramp rate of 0.1 °C/min at a chamber pressure of 0.05 mbar and then maintaining temperature for 4 h.

At the end of the freeze-drying cycle, vials were closed under vacuum in the drying chamber before unloading.

2.4. Tableting of FD powders

Tablets were manufactured with a compaction simulator « Styl'One Evolution » (Medelpharm) equipped with euro B flat punches of 6 mm diameter. All compaction experiments were performed under controlled temperature (22 °C) and relative humidity conditions (30–40 %). Before compression, FD cakes were gently re-dispersed to a powdered state using a mortar and pestle. For the experiments, the die filling height was adjusted to 10 mm and the powder was manually inserted into the die. Manual feeding is quite far from an automated feeding which is required for a high-speed tableting press but this step is out of the scope of this study. All cycles were performed at a low compression speed of 2 % (corresponding to cycle time around 2 s) and an external lubrication system with magnesium stearate was used to lubricate the punches and the die. FD powders were compacted using the force-driven mode of Analis software at three different levels: 0.71 kN, 1.42 kN and 2,84 kN (namely respective compression pressures of 25, 50, 100 MPa considering the chosen punches). The tablets have approximate weights of 50 mg (one vial per tablet).

2.5. Physico-chemical and mechanical properties of FD and tableted samples

2.5.1. Scanning electron microscopy (SEM)

FD powders were observed by SEM using a tabletop microscope (Hitachi TM3000, Japan). A sharp blade was used to cut thin slices of the FD samples. The cut FD samples were then fixed to aluminum stubs with double-sided carbon tapes in order to visualize their morphology.

2.5.2. Differential scanning calorimetry

The thermal properties of the samples were assessed using differential scanning calorimetry (DSC) with a DSC3 instrument (Mettler Toledo, Switzerland). The samples were prepared in sealed aluminum capsules of 100 μL. In the case of aqueous formulations, the glass transition temperatures of the maximally freeze-concentrated solution (T'_g) were determined by cooling from 25 °C to − 50 °C at a rate of 1 °C/min, followed by heating to 25 °C at a rate of 10 °C/min. To determine the glass transition temperature (T_g) of solid samples (FD or tablets), experiments were conducted by heating the samples from 10 °C to 230 °C at a rate of 10 °C/min. Midpoint T'_g and T_g of each sample were determined from thermograms using STAR^e analysis software.

2.5.3. Relative humidity

Residual moisture content in the FD samples was measured by Karl Fisher Titration method using a C20S coulometric KF titrator (Mettler Toledo, Greinfensee, Switzerland). Samples (approximately 50 mg), kept under vacuum until titration, were resuspended in 1 mL of anhydrous methanol (Acroseal®) and the entire volume was injected into the system and titrated with Riedel-de Haen Hydranal Composite 2 reagent (Hoechst Celanese Corp.). Experiments were carried out in triplicate and the water content in % w/w was expressed as mean ± SD.

2.5.4. X-ray diffraction (XRD)

Commercial dissacharides powders (cf. **2.1**), FD and tablet samples were analyzed with a benchtop powder X-ray diffractometer (MiniFlex2, Rigaku) with Cu Kα radiation (30 kV, 15 mA). Each scan was performed in the 2θ range from 0 to 60° with a scan speed of 5°/min.

2.5.5. Tablets tensile strength measurements

A diametral compression test was performed on tablet samples using a TA.HD. plus texture analyzer (TA.HD, Stable microsystems, Surrey, United Kingdom) to assess the tabletability profile of each FD powder formulation (containing either trehalose or sucrose as main excipient). Tablet dimensions (diameter and thickness) were measured using calipers and then compacts were diametrically compressed between two flat surfaces at a constant speed of 0.1 mm.s^{-1} until the failure. Experiments were carried out in triplicate.

Tensile strength (σ) was defined as:

$$\sigma(MPa) = \frac{2F}{\pi dh}$$

where F = breaking strength (N), d = tablet diameter (mm) and h = tablet thickness (mm).

2.6. Indirect enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA detection method was used to evaluate the binding activity of TAT-1 mAb following freeze-drying and tableting processes. Nunc Maxisorp 96-well plates (Thermo Fisher scientific) were coated with 1 μg of tubulin per well in 50 mM bicarbonate buffer pH 9.6 at 4 °C overnight (100 μL/well). The next day, wells were blocked with PBS/gelatin 0.2 % (w/v) (200 μL/well) for 30 min at 37 °C. The blocking buffer was removed and wells were washed 3 times with 300 μL of PBS/Tween-20 0.05 % solution. FD and tablets samples were reconstituted with 1 mL of PBS and sample's mAb quantity was determined by the Bradford method (Protein assay, BioRad) using bovine gamma globulin (Thermo Fisher scientific) as standard reference for the calibration curve. The washing buffer was removed and 100 μL of each sample diluted to a final mAb concentration of 150 ng/mL was added in the wells and incubated for 1 h30 at 37 °C, in triplicate. After a new washing step, plates were incubated for 1 h at 37 °C with 100 μL/well of horseradish peroxidase-conjugated sheep anti-mouse IgG (H + L) (Jackson Immuno Research, #515–035-062) diluted 1:5000. Plates were washed and 100 μL/well of a solution of citric buffer at 50 mM pH 4 containing H_2O_2 (0.03 % (v/v)) and ABTS substrate (2,2′-azino-bis(3-éthylbenzthiazoline-6-sulfonic acid) (40 μM) was added and the plates were incubated for 20 min. Absorbance was measured at 405 nm in a plate reader.

2.7. Surface plasmon resonance (SPR)

Surface plasmon resonance (SPR) was used to determine the binding affinity of mAb's Fab and Fc fragments after each process (FD and tableting). Experiments were conducted at 25 °C using a Biacore T200

system (GE Healthcare Life Science) with a pre-filtered and degassed PBS/Tween 20 running buffer (0.05 % (v/v)). Proteins and peptides used as ligands were prepared at concentrations of 10–20 μg/mL in sodium acetate buffer at pH 5–7. The ligand solutions were injected onto the surface of a flow cell in a CM5 chip (GE Healthcare Life Science) and ligands were immobilized by amine coupling according to manufacturer's recommendations. One track on the chip was left blank for double referencing of the sensorgrams. Antibodies were dialyzed against the running buffer and injected onto their respective targets using a Single Cycle Kinetic (SCK) approach. This method involved sequential injection at increasing concentrations without chip regeneration between injections. Antibodies were injected for 60 s at three increasing concentrations (22, 66, 200 mM) with a dissociation time of 240 s. After each SCK cycle, the chip was regenerated for 30 s with 10 mM NaOH solution. Sensorgrams were analyzed using Biacore T200 2.0 evaluation software (BiacoreTM). The association (k_a) and dissociation (k_d) rate constants were determined by directly fitting the sensorgram curves to a 1:1 Langmuir interaction model. The equilibrium dissociation constant, K_D, was calculated as k_d/k_a.

2.8. Ultracentrifugation

Sedimentation velocity experiments were performed on an analytical ultracentrifuge Beckman XL-I (Beckman Coulter, Palo Alto, USA) with an An-50Ti rotor at 20 °C. The FD and tablet samples reconstituted in PBS and aqueous samples of mAb in PBS buffer with or without excipients, were prepared at a final concentration of 0.5 mg/mL. These preparations were done approximately 30 min prior to the experiments. For all experiments, 400 μL of samples and 410 μL of their respective buffer references were loaded into two-sector 12 mm path-length Epon charcoal-filled centerpiece and centrifuged at 42 000 rpm. Absorbances at 280 nm were collected every 10 min. Buffer viscosities and densities were measured experimentally with an Anton Paar microviscosimeter and density meter (DMA 4500). Data of sedimentation velocity distributions were analyzed using SEDFIT software.

3. Results and discussion

3.1. Design and characterization of a FD formulation suitable for the development of an antibody tablet formulation

3.1.1. Formulation and process parameters selection

An appropriate selection of excipients for the formulation of FD biological molecules is crucial since excipients must address two key objectives: ensuring the physico-chemical stability of the active substance, but also, ensuring the obtention of a product that meets the required quality attributes for lyophilized pharmaceutical products (macroscopic appearance, residual water content, stability, dissolution time, etc.) (Hsein et al., 2022). Generally, formulations of FD proteins contain a combination of excipients including a buffering agent, a bulking agent, a surfactant and a stabilizing agent (or bioprotectant) (Cui et al., 2017; Strickley and Lambert, 2021). In many cases, the stabilizing agent is carefully chosen to fulfill both protective and bulking agent roles. In this study, two different formulations were selected and tested for the freeze-drying of mAbs: one containing trehalose (5 % w/v) with Tween-20 (0.005 % v/v), and the second containing sucrose (5 % w/v) with Tween 20 (0.005 % v/v). This choice was based on a literature review that highlighted the widespread use of these excipients in FD antibodies formulations. Sucrose and trehalose, both disaccharides, were chosen for their well-established roles as effective protective agents during freezing (cryoprotectant) and drying steps (lyoprotectant) (Crowe et al., 1987, 1984; Sun et al., 1996). Polysorbate 20 was chosen as a surfactant known to limit molecular aggregation of proteins during the freeze-drying process.

Although freeze-drying is the method of choice for improving the stability of biopharmaceuticals, it remains a critical process that exposes biomolecules to significant stresses during freezing and drying stages (Wang, 2000). In addition to excipients, a meticulous selection of operational parameters is therefore also essential to preserve the biological integrity of therapeutic molecules during the process. Among the major risks, the collapse of the dry matrix during drying is known to potentially adversely affect the properties of FD materials (increase in residual humidity, risk of aggregation). Despite an increasing number of studies indicating that collapse is not necessarily detrimental to protein stability (Schersch et al., 2010; Wang et al., 2004), it is conventionally accepted to prevent collapse by adjusting the shelf temperature during the sublimation to maintain the product temperature below its critical collapse temperature (Tc) (Tang and Pikal, 2004). Tc is generally a few degrees below the glass transition temperature (T'_g) of the system. T'_g values of −34 °C and −36 °C were respectively determined for the selected trehalose and sucrose formulations using DSC analysis on placebo samples. Using our freeze-drying operational protocol (detailed in section 2.3), a shelf temperature of −20 °C and a pressure of 0.1 mbar were implemented during the sublimation step to fulfill these criteria.

As expected, the obtained cakes did not visually exhibit signs of collapse or cracks. The internal morphology of FD samples was also observed through scanning electron microscopy (SEM). As shown in Fig. 1, SEM images revealed a porous microstructure characterized by an alveolar organization with sheets and pores similar to literature-derived data (Hedberg et al., 2019). Furthermore, this observation did not reveal any localized micro-collapse within the internal structure of the dry matrix.

Placebo FD samples were also characterized in order to study their physico-chemical properties. The residual moisture (RM) content of the obtained dried formulations has been measured using the Karl Fischer method. The percentage of RM (or RM %) for FD trehalose and FD sucrose were 0.54 ± 0.12 % and 1.33 ± 0.12 %, respectively. These values are acceptable, as a percentage of RM ≤ 1 % or ranging between 1 and 3 % (depending on the type of the protein) is commonly recommended to ensure biopharmaceuticals stability when formulated in a dried form (Horn et al., 2018; Schneid et al., 2011).

Subsequently, XRD experiments were conducted on both dried placebo formulations in order to analyze the physical state of the obtained FD systems. The results presented in Fig. 2 showed a crystalline state for both trehalose and sucrose before freeze-drying and that both formulations were entirely amorphous after freeze-drying. The amorphous structure of FD samples is the main reason why trehalose and sucrose were incorporated into the formulations. Indeed, the ability of these disaccharides to form an amorphous matrix constitutes a robust hypothesis to explain their role as bioprotective agents during freeze-drying (Sun et al., 1996). To gain further insights into the physical state of the obtained FD systems, DSC analysis were performed. The thermal analysis highlighted a glass transition temperature (Tg) for FD formulations. The glass transition occurs within a temperature range where molecular mobility significantly increases, which could potentially compromise the stability of the bioproduct if this temperature is reached. Therefore, this characteristic is an important factor to consider as it provides insights into the storage conditions of biological molecules formulated in the dry state. Tg values of 100 ± 0.8 °C and 62.4 ± 0.4 °C were measured for FD trehalose and FD sucrose placebo samples respectively. These values are consistent with literature data for similar formulations (Baheti et al., 2010; Bjelošević et al., 2020). The detection of a glass transition phenomenon during DSC analysis confirms that the solid systems obtained after freeze-drying were in an amorphous state.

Overall, these analyses confirmed that with the operational and formulation parameters used, the FD samples obtained from both formulations met the required quality criteria to theoretically ensure the stability of biomolecules such as antibodies during and after freeze-drying.

3.1.2. Tableting behavior of selected FD formulations

Tableting behavior of FD powders was evaluated by studying their

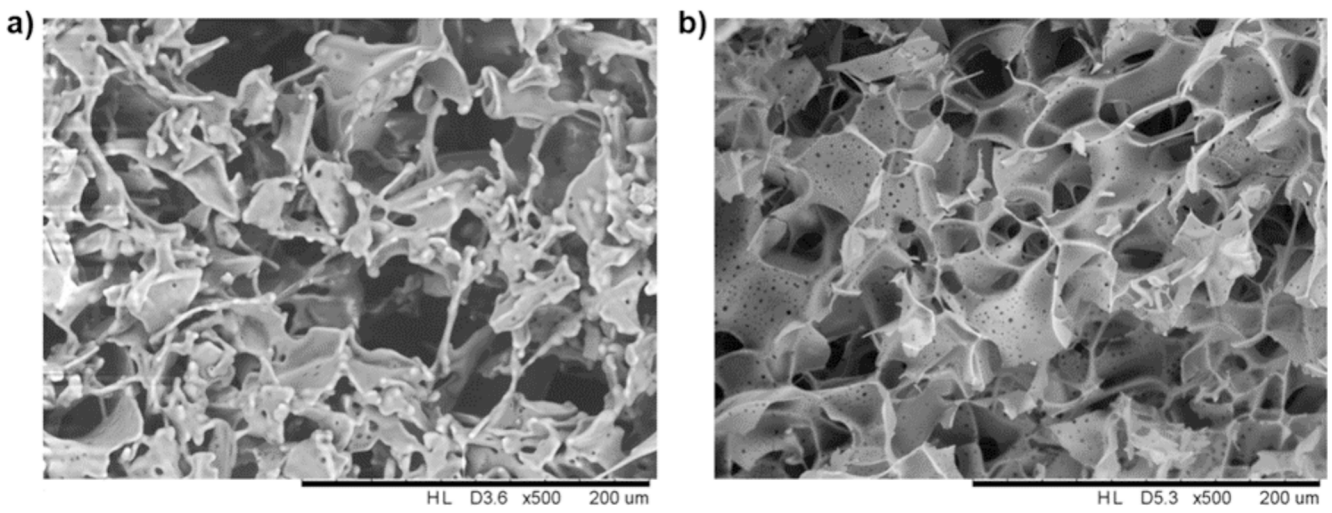

Fig. 1. SEM images of FD sucrose (a) and trehalose (b) cakes structure (500 × magnification).

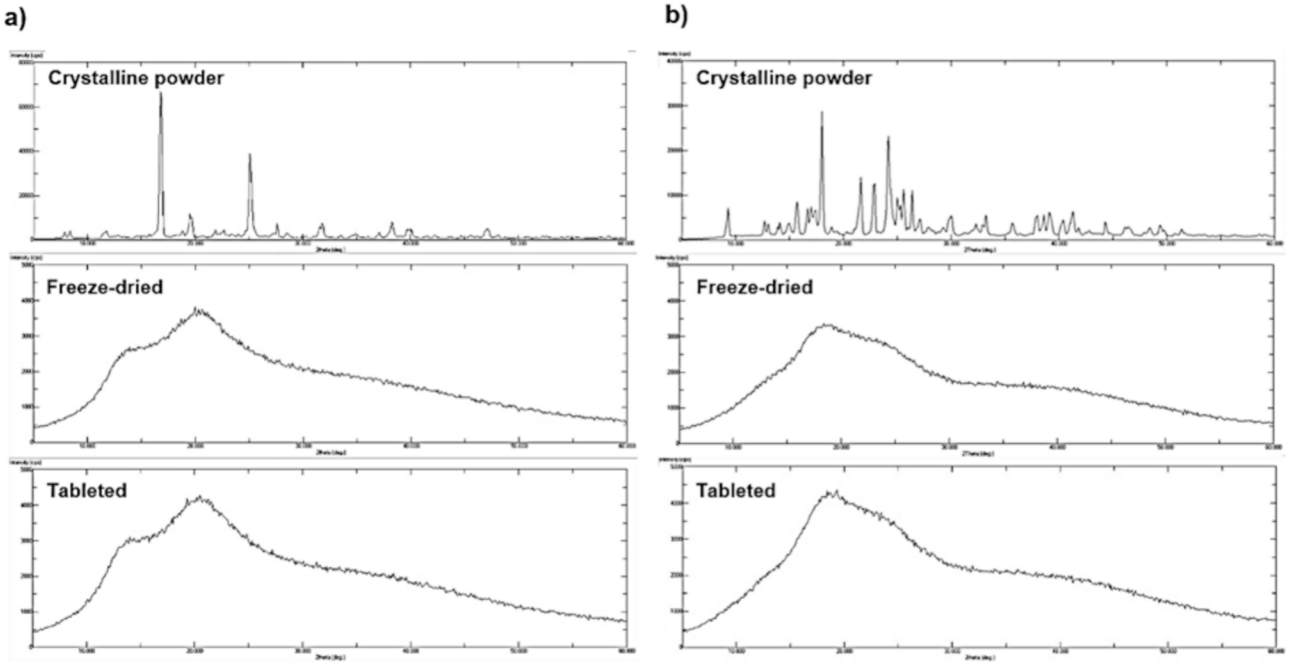

Fig. 2. XRD diffractograms of crystalline, FD and tableted (25 MPa) sucrose (a) and trehalose (b) powders.

tabletability profile. Tabletability is a crucial parameter to consider because it makes it possible to determine the pressure range necessary to form a tablet with satisfactory mechanical strength. Tabletability profile was obtained by plotting the tensile strength of the compacts as a function of applied pressures. Typically, a target mechanical strength in the range of 1–2 MPa for a tablet is considered suitable to ensure its integrity throughout its lifecycle, from compaction to its use by the patient. The range of applied pressure (25–100 MPa) chosen for this characterization was based on the recent study of Hsein et al. and Madi et al, who demonstrated that FD trehalose exhibits very singular compaction properties (Hsein et al., 2023; Madi et al., 2024). They demonstrated that FD trehalose powder is cohesive at very low compaction pressure (25 MPa) compared to other commonly used excipients, such as crystallized lactose or mannitol while having a good compressibility profile. This behavior was associated to the special texture and to the amorphous state of FD dissacharide formulations. As shown in Fig. 3, our results corroborate with these findings, wherein tablets with respective acceptable average tensile strengths of 1.8 and 2.5 MPa for FD trehalose and FD sucrose placebo formulations were obtained at a compaction pressure of 25 MPa.

In the context of this study, the distinctive tabletability profile linked to our chosen FD formulations was particularly advantageous. It indicates that low compaction pressures, and thus minimal mechanical stresses applied to the antibody-containing dried matrix, are sufficient to obtain tablets with a satisfactory mechanical strength.

3.2. *Influence of tableting on mAb biological integrity*

3.2.1. *Effect of compaction on mAb's binding abilities*

To investigate if tableting has a potential impact on the biological integrity of mAbs, ELISA experiments were first conducted in order to measure the binding activity of the mAb before and after compaction under increasing pressures (25, 50, and 100 MPa). In first intention, the quantity of TAT-1 in aqueous formulations for freeze-drying was chosen

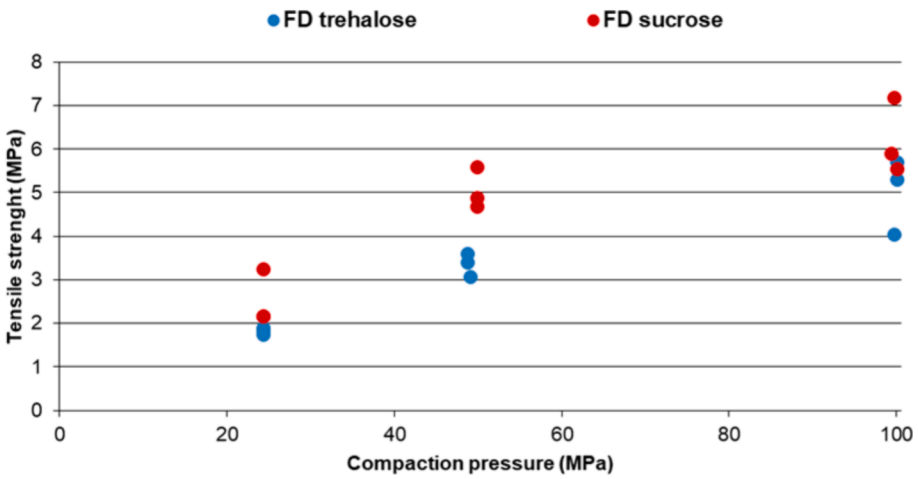

Fig. 3. Tabletability profiles of FD sucrose and FD trehalose formulations showing the evolution of tensile strength as a function of applied axial compaction pressure.

to achieve tablets, each containing 100 µg of the antibody. It is important to mention that a coefficient of variation (CV) up to 10 % was calculated for the triplicates in our experimental ELISA conditions. It is well accepted that a CV around 10 %-15 % is still satisfactory using ELISA (Minic et al., 2020). Therefore, a variation in absorbance values < 10 % was not regarded as a significant modification of the binding activity in our study. For the analyse, absorbances obtained with the mAb before each process (FD + tableting) (i.e in the liquid form) were normalized and expressed as 100 % of the enzymatic activity (control). To specifically determine the influence of the tableting process, a reference with the FD powders was also included in the study.

The obtained results (Fig. 4) firstly show that the binding activity of the mAb, measured from reconstituted FD samples, is not significantly altered compared to the control condition for both trehalose (102 %) and sucrose formulations (98 %). Concerning reconstituted tablets samples, the results also indicate that there are no changes in the binding activity of the mAb after compression under each tested compaction pressure when formulated with both trehalose (enzymatic activity of 104 %, 110 %, and 105 % measured at 25, 50, and 100 MPa respectively) and sucrose formulations (101 %, 103 %, and 96 % at 25, 50, and 100 MPa respectively) compared to the control condition. Thus, the presented results demonstrate that with the selected formulations and operationnal used conditions, the binding activity of the mAb is preserved after freeze-drying. In addition, the obtained results also demonstrate that compaction of FD mAbs at compression pressure up to 100 MPa is not detrimental for the mAb's binding activity when formulating with sucrose and trehalose.

To go further, ELISA method was also used to evaluate the potential influence of increasing mAb quantities in the tablets. Tablets formulated from both disaccharide formulations, each containing 500 µg of the mAb, were thus compacted at the target pressure of 25 MPa (that was previously identified as sufficient to obtain a tablet with satisfactory mechanical properties (see Fig. 3)). Similar to the previous results, the obtained data (Fig. 5) indicate that for each formulation, the binding activity of the mAb is not significantly altered neither after freeze-drying (enzymatic activity varying from 104% for FD trehalose to 95 % for FD sucrose) nor after tableting under compaction pressure of 25 MPa (94 % for trehalose tablet and 93 % for sucrose tablet) compared to control samples when formulating at higher concentration.

To gain a more precise understanding of the potential impact of tableting on mAb binding capabilities, Surface Plasmon Resonance (SPR) experiments were conducted to accurately assess the binding affinity of the mAb before and after tableting when formulated in the same last conditions (500 µg/tablet, 25 MPa). Given the essential roles of both Fab and Fc antibody's fragments in ensuring its complete biological functionality, the TAT-1 mab's binding affinity was measured for the Fab fragments (against tubulin) as well as for the Fc fragment by examining its interaction with streptococcal protein G (known to present affinity for immunoglobulin Fc fragments). For both formulations, the obtained binding kinetic profiles of the mAb after reconstitution of FD

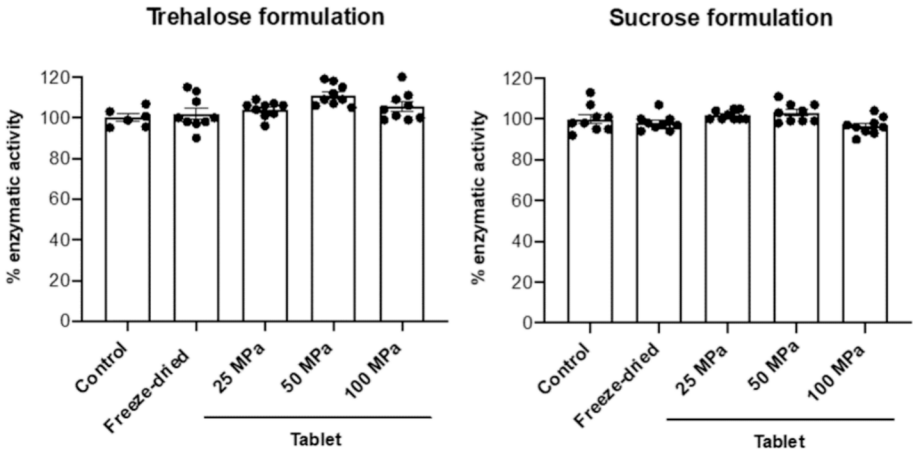

Fig. 4. Study of the influence of the compaction pressure (ranging from 25 to 100 MPa) and the formulation on the binding activity of the TAT-1 mAb (100 µg/tablet) using indirect ELISA.

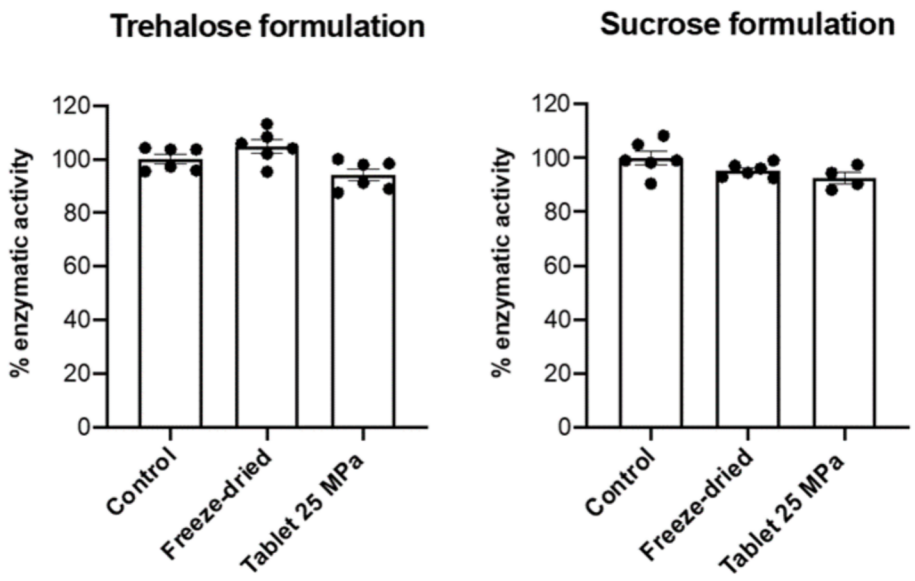

Fig. 5. Study of the influence of tableting (25 MPa) and formulation on the binding activity of the TAT-1 mAb when formulated at 500 μg/tablet using indirect ELISA.

samples or tablets are presented in Fig. 6. Results showed (Table 1) that for each formulation, K_D values measured for both types of fragments are substantially identical after freeze-drying and compaction processes. This result consolidates the previous results obtained from ELISA experiments but also confirms that with the selected operating and formulation conditions, the binding capabilities of the mAb are fully preserved during its development into tablet form.

These results differ from previous findings by Lu et al., where a significant loss in binding activity was systematically observed by ELISA when compaction pressure as low as 50 MPa were applied on a formulation of FD polyclonal antibodies (Lu et al., 2021). In the same time, these results corroborate with the results obtained by Wei et al.(Wei et al., 2019) who demonstrated that the compaction of lysozyme powder, maintained in an amorphous state even under high compaction pressure, was not detrimental to the enzymatic activity of the protein. It is indeed well known that compaction can destabilize the physical state of the granular system, leading to the recrystallization of an initially amorphous structure (Rams-Baron et al., 2018). Here, our DSC results have confirmed that FD samples from the both tested disaccharide formulations remained amorphous after compaction at 25 MPa (see Fig. 2). In a recent study, Hsein et al. also demonstrated the preservation of the amorphous state of the tableted FD trehalose powder even under higher compaction pressures (Hsein et al., 2023). Considering the crucial role of the amorphous structural state in preserving the biological properties of solid-state formulated proteins, it is reasonable to speculate that maintaining this physical state after compression may contribute to maintain the binding activity of the mAb after tableting.

We note that significantly lower Tg values were measured by DSC for the tablets (73 °C for trehalose-based tablets and 48 °C for sucrose-based tablets) compared to their corresponding FD powder (100 °C for FD trehalose and 62 °C for FD sucrose). It is well known that Tg values decrease with the increase of residual humidity (Roe and Labuza, 2005). This decrease in Tg values for tablets can thus probably be explained by the absorption of water by the powders during their handling (grinding, deposition into the matrix) before compaction, as a residual moisture content of 4 % has been determined for the FD tablets obtained from both the trehalose (Hsein et al., 2023) and sucrose formulations.

As no significant difference was observed between formulations containing sucrose or trehalose to preserve the biological integrity of the mAbs, only the trehalose formulation—with the highest glass transition temperature (Tg) and, consequently, better stability and storage conditions—was selected for further characterizations. Moreover, since the compaction pressure of 25 MPa was demonstrated to be sufficient for obtaining tablets with satisfactory mechanical strength using FD trehalose powder, the effect of tableting was only investigated using this compaction pressure for subsequent investigations.

3.3. *Effect of tableting on mAb aggregation*

It has been demonstrated that the tableting of proteins formulated into solid-state can induce their aggregation (Wei et al., 2019). Aggregation is a significant obstacle in the pharmaceutical development of therapeutic antibodies, as it may compromise their biological function. Aggregation of mAb has also been associated to robust and undesirable immunogenic reactions (Shomali et al., 2015; Uchino et al., 2017). Analytical ultracentrifugation coupled to absorbance detection was used to compare the rate of mAbs aggregates before and after tableting.

As shown in Fig. 7**a**, the aqueous trehalose-formulation of the mAb contains a large majority of species with a sedimentation coefficient of 6.4 S ($S_{20,W}$ = 7 S). This peak corresponds to the monomeric form of the mAb and represents 98 % of all protein species, while small aggregates account for only 2 %. From the reconstituted FD sample, similar amounts of monomers (97.6 %) and small aggregates (2.4 %) were identified (Fig. 7**b**), indicating no distinctive impact of the freeze-drying process on mAb aggregation under our operational conditions. Compared to the control and FD conditions, only a minor loss of monomeric content (− 4 %) was observed in the reconstituted tablet sample (Fig. 7**c**). Considering the ELISA and SPR results described before (Fig. 5**,** Table 1), it is possible to conclude that a 4 % increase in the percentage of small aggregates after compression did not affect the antibodies binding properties.

Interestingly, we also observed that the sedimentation coefficient of the majoritary peak decreases between the initial solution ($S_{20,W}$ = 7.05S) and the reconstituted FD and tablet samples (respectively $S_{20,W}$ = 6.6 and $S_{20,W}$ = 6.7). This difference could be correlated with a more elongated shape of the mAb in the reconstituted FD and tablet samples compared to the control condition. Indeed, the calculated mAb's frictional coefficient ratio f/f0 in the control condition is 1.42, it increases to 1.55 in the FD and tablet reconstituted samples, respectively.

However, additional investigations have revealed a similar increase in the coefficient of friction of the mAb when only excipients (trehalose, polysorbate 20) are added in the initial mAb solution (1.54).

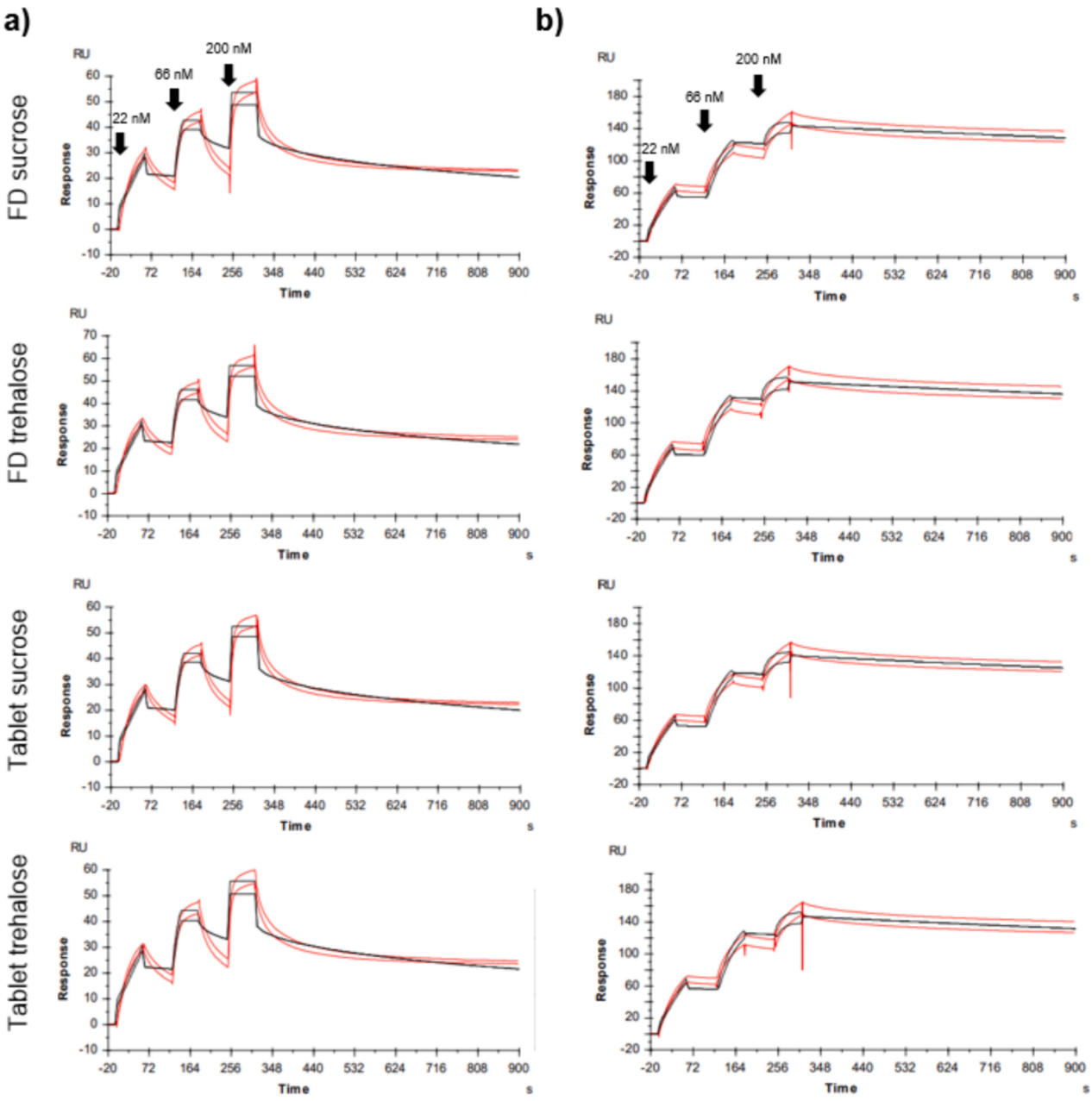

Fig. 6. Kinetic analysis by surface plasmon resonance of the binding of the mAb TAT-1 to tubulin (a) and to protein G (b) when formulated with trehalose or sucrose after freeze-drying and after tableting (25 MPa).

Table 1
Association (k_a), dissociation (k_d) and equilibrium dissociation (K_D) constants of the TAT-1 mAb with tubulin and protein G after FD and tableting (25 MPa) processes measured by SPR.

		Sucrose		Trehalose	
		FD	Tablet	FD	Tablet
Tubuline	ka (1/Ms)	$8{,}21.10^{8}$	$9{,}36.10^{8}$	$1{,}52.10^{9}$	$1{,}07.10^{9}$
	kd (1/s)	$5{,}22.10^{-1}$	$6{,}01.10^{-1}$	1,06	$6{,}53.10^{-1}$
	K_D (M)	**$6{,}36.10^{-10}$**	**$6{,}42.10^{-10}$**	**$6{,}97.10^{-10}$**	**$6{,}1.10^{-10}$**
Protein G	ka (1/Ms)	$3{,}71.10^{5}$	$3{,}62.10^{5}$	$3{,}86.10^{5}$	$3{,}74.10^{5}$
	kd (1/s)	$1{,}87.10^{-4}$	$1{,}95.10^{-4}$	$1{,}79.10^{-4}$	$1{,}81.10^{-4}$
	K_D (M)	**$5{,}04.10^{-10}$**	**$5{,}39.10^{-10}$**	**$4{,}64.10^{-10}$**	**$4{,}84.10^{-10}$**

Consequently, this result suggests that the elongation of the mAb is probably induced by the excipients rather than by the processes.

3.4. Influence of tableting on the mAb stability during storage

Finally, the stability of the tableted TAT-1 mAb was assessed by measuring its binding activity via ELISA from reconstituted tablet following storage at different temperatures (5, 25 and 40 °C) over a 6-month period. A comparison with that of the FD samples stored at 4 °C over the same duration was made. As it is well known that a high level of residual moisture can compromise protein stability, all tablets were stored in glass vials which were closed in a glove box under controlled humidity condition (7–10 %) during the duration of the test.

As presented in Fig. 8, the results reveal that the binding ability of the mAb is fully preserved following storage at 4 °C and 25 °C for 6 months. However, during the accelerated stability study (40 °C), it was noted

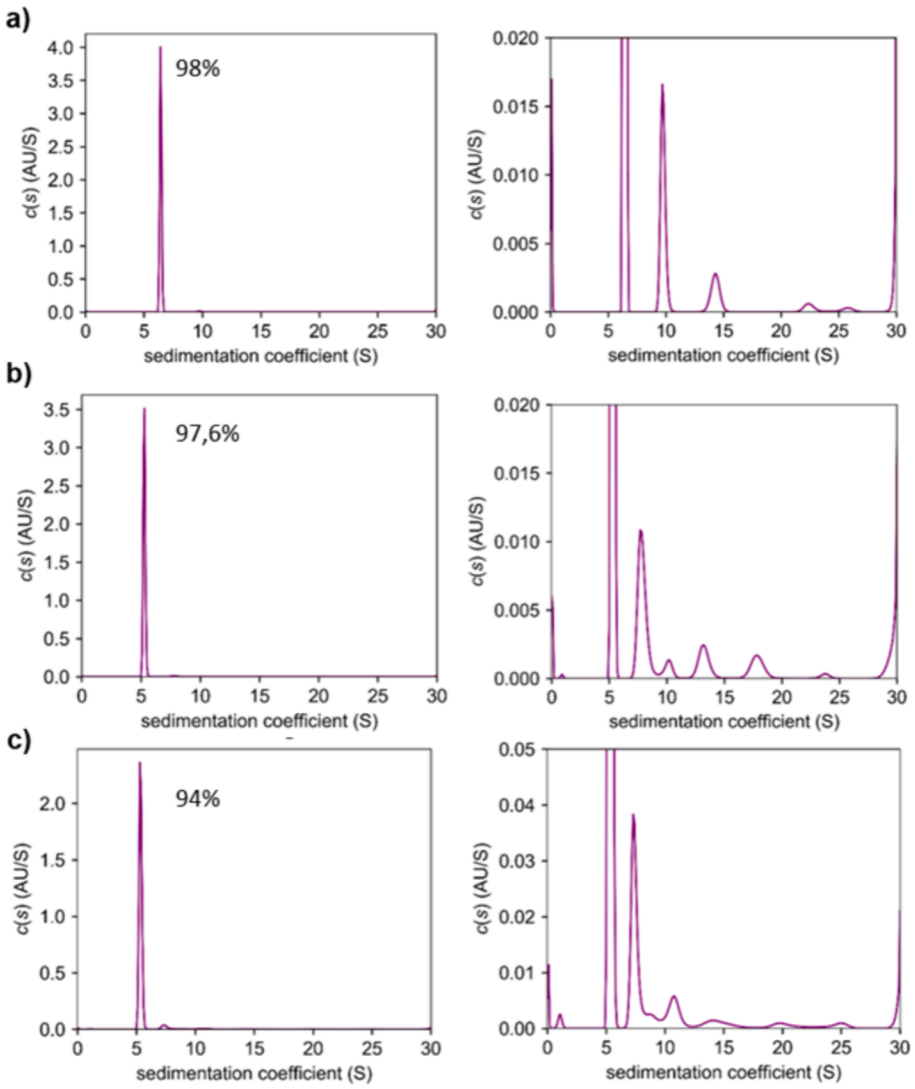

Fig. 7. Distribution curves (left images) and zoom of the distribution curves (right image) of protein species contained in the initial solution of the mAb TAT-1 (a), after FD reconstitution (b) and after tablet reconstitution (c) obtained by analytical ultracentrifugation using absorbance detector (280 nm).

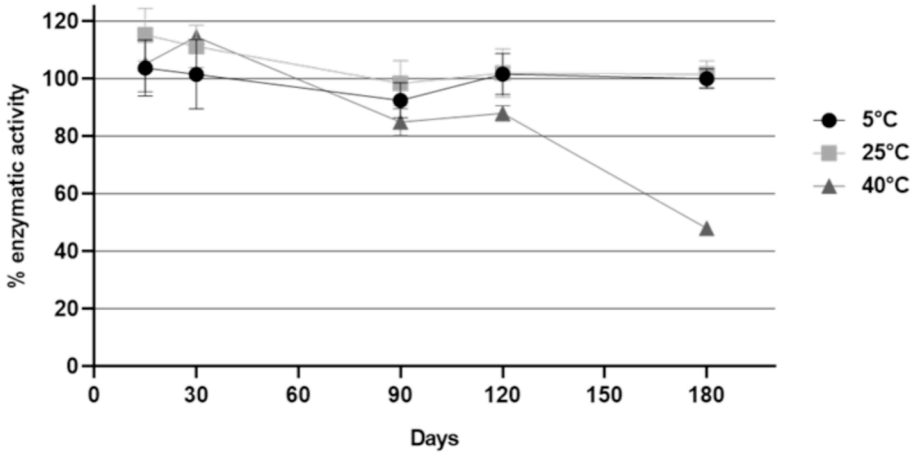

Fig. 8. Stability study of tableted TAT-1 mAb during storage at 5, 25 and 40 °C for 6 months evaluated by indirect ELISA.

that the binding activity of the mAb decreased significantly after 3 months, gradually reaching a 52 % loss by the end of the 6-month period.

4. Conclusion

This study demonstrates the feasibility of developing antibodies in tablet form while maintaining their biological activity and stability for at least 6 months at room temperature. By selecting two lyophilized antibody formulations with either trehalose or sucrose as the main excipients, the obtained results showed that the compaction of the obtained powders at a low pressure of 25 MPa is sufficient to achieve tablets with good mechanical properties. Furthermore, compressing them up to 100 MPa has been shown to have no detrimental impact on the antibody's binding capacities. More specifically, we observed that for both formulations, the binding affinity of the Fc fragment (essential when the therapeutic strategy relies on antibody-dependent cellular functions) as well as of the Fab fragments (crucial for neutralizing or opsonizing mAbs effects) was fully maintained after tablet reconstitution when tableted under the compaction pressure of 25 MPa. Finally, the results from ultracentrifugation assays did not reveal a significant increase in the aggregate rate after tablet reconstitution.

To our knowledge, this is the first study that explicitly evaluates the effect of tableting on the binding capacities and affinity constants of mAbs under well-defined compression conditions (formulation and process parameters).

CRediT authorship contribution statement

Julie Auffray: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Hassana Hsein:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Nicolas Biteau:** Validation, Methodology, Investigation. **Christophe Velours:** Writing – review & editing, Validation, Methodology, Investigation. **Thierry Noel:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Pierre Tchoreloff:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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

The data that has been used is confidential.

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