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Monoclonal antibody formulation manufactured by high-speed electrospinning

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ABSTRACT

Solid formulations of monoclonal antibodies present several advantages, such as improved stability and increased shelf-life as well as simpler storage and transportation. In this study, we present a gentle drying technology for monoclonal antibodies, applying the water soluble 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as matrix, to prepare a solid reconstitution dosage form. High-speed electrospinning of an aqueous infliximab-containing HP-β-CD solution was carried out at 25 °C resulting in fibers with an average diameter of 2.5 μm. The mAb-loaded electrospun fibers were successful to preserve the stability of infliximab in solid form. The results of size exclusion chromatography and gel electrophoresis indicated no significant increase in aggregate formation during the electrospinning process compared to the initial matrix solution. The binding activity of infliximab was preserved during electrospinning compared to the reference liquid formulation. Due to the enhanced surface area, excellent reconstitution capability, i.e. clear solution within 2 min without any vigorous mixing, could be achieved in a small-scale reconstitution test. The results of this work demonstrate that high-speed electrospinning is a very promising technique to manufacture the solid formulation of monoclonal antibodies for applications such as fast reconstitutable powders.

1. Introduction

Monoclonal antibodies (mAbs) are primarily administered parenterally by intravenous, subcutaneous or intramuscular routes, where the final dosage form requires a liquid-based formulation (Shire, 2015). Liquid drug products involve the advantages of low cost, fast development process and convenient preparation for the administration, making them attractive from the manufacturing and marketing perspectives (Uchiyama, 2014). However, in the presence of water mAbs can undergo a multitude of hydrolytically driven degradation routes such as oxidation, deamidation, fragmentation or aggregation (Chang and Hershenson, 2002; Daugherty and Mrsny, 2006; Harn et al., 2010). In particular, the control and mitigation of aggregate formation is vitally important, as it can lead to further degradation and loss of therapeutic efficacy, reduced reproducibility or unwanted immune reactions in patients (Rosenberg, 2006; Cordoba-Rodriguez, 2008; Lowe et al., 2011).

In the solid state, therapeutic mAbs have significantly improved stability and prolonged shelf life because molecular mobility is restricted and hydrolytic reactions are minimized (Abdul-Fattah and Truong, 2010). Furthermore, solid formulations enable decreased storage space and a potentially greater tolerance for room-temperature storage, resulting in simpler packaging and transportation, and avoidance of the freeze–thaw cycles. Currently, the most widely used technique for solidification of mAb pharmaceuticals for parenteral usage is freeze drying, though attempts have been made to apply spray drying with some success (Batens et al., 2018; Bowen et al., 2013). Despite the promising results, it is still early to claim that spray drying is a viable alternative drying process among mAbs, as the technology has several limitations like the employed high drying temperature and shear forces (Vass et al., 2019). During freeze drying, the therapeutic antibodies are exposed to a combination of two distinct stresses: freezing and dehydration (Wang, 2000). In order to minimize the degradation of proteins and preserve their biological activity, the addition of stabilizing excipients (cryoprotectants and lyoprotectants) could be beneficial. The most commonly employed stabilizers are sugars, surfactants and amino acids (Angkawinitwong et al., 2015; Emami et al., 2018; Chang et al., 2005). But even

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though the stress imposed during drying process can be well controlled by these protective agents, freeze drying remains a high energy- and time consuming batch technology involving complex and multi-step processes (Vass et al., 2019). However, numerous studies are focusing on innovative continuous freeze drying technologies which can offer a gentle scalable energy-efficient process with improved productivity (De Meyer et al., 2015; Capozzi et al., 2019).

Electrostatic spinning (Electrospinning, ES) is a new drying technology for the pharmaceutical industry, where solvent evaporation is carried out at ambient conditions. The technology utilizes electrostatic forces to produce ultrathin fibers with diameters in the nm to μm range (Huang et al., 2003; Li and Xia, 2004; Greiner and Wendorff, 2007). Compared to freeze drying, ES can be operated in continuous mode with low energy consumption, where the generated liquid jets dry extremely rapidly ($t < 0.1$ s) due to their huge surface area. In the last decade, ES technology has been successfully applied for the solidification of various bioactive agents such as hormones, enzymes, proteins, oligonucleotides and living cells (Zussman, 2011; Jiang et al., 2014; Hu et al., 2014; Nagy et al., 2014; Wagner et al., 2015; Choi et al., 2015; Hirsch et al., 2019; Angkawinitwong et al., 2017). In 2017, Angkawinitwong et al. (2017) first reported about a sustained release encapsulated form of bevacizumab prepared by coaxial ES technology for the treatment of age-related macular degeneration. The mAb-loaded core–shell nanofibers were successfully fabricated without inducing any protein degradation. Despite increasing attention to ES as a potentially novel drying technology, its commercial application remains limited mainly due to the relatively low productivity of the technology and low drug loadings in the formulations. For example, there was a study where a promising electropsun formulation was developed for selective intravaginal drug release using a pH-responsive matrix system (Tyo et al., 2019). However, the production was performed by a laboratory-scale device with a flow rate of 0.8 mL/h achieving low productivity (<120 mg/h), which does not satisfy industrial requirements. Moreover, in the case of sensitive biopharmaceuticals, water is the most preferable solvent, which (due to its low volatility) further decreases the throughput of the technology compared to organic solvents. To meet the industrial needs of high productivity, high-speed electrospinning (HSES) technology has been developed (Nagy et al., 2015). The technology utilizes high voltage and a rotating disk-shaped spinneret with orifices, where the combination of electrostatic and centrifugal forces results in increased throughput.

Cyclodextrins (CDs) as widely used excipients in pharmaceutical formulations, have been shown to be able to inhibit or slow down the aggregation of therapeutic proteins (Davis and Brewster, 2004; Otzen et al., 2002; Serno et al., 2011). Moreover, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) was approved for parenteral use with doses up to 16 g/day (Davis and Brewster, 2004). Thus, CDs have been applied as protective excipients for the preparation of freeze-dried (Iwai et al., 2007; Faghihi et al., 2015) and spray dried (Branchu et al., 1999; Ramezani et al., 2017) protein formulations. A recent study demonstrated that the use of HP-β-CD in freeze dried mAb formulations was beneficial to reduce the duration of freezing and drying processes, furthermore ensured room temperature storage stability up to 9 months (Haeuser et al., 2020). Moreover, it was reported that HP-β-CD at a remarkably low concentration (<0.4 w/w%) had similar surface activity characteristics to nonionic surfactants and significantly helped to suppress the agitation-induced aggregation of a mAb in aqueous solution (Serno et al., 2010). Besides that, CDs are capable of self-assembly in high concentrated solutions and several investigations have successfully applied them as a non-polymeric matrix system to fabricate CD-based nanofibers by electrospinning technology (Celebioglu and Uyar, 2012; Vass et al., 2019).

The aim of this research was to evaluate the feasibility of HSES as an alternative drying technology to produce a dried formulation of a monoclonal antibody. Infliximab was selected as the model active mAb. It is a chimeric monoclonal antibody with a molecular weight of 144 kDa that inhibits the human tumor necrosis factor-alpha (TNF alpha). It was approved for the treatment of Crohn's disease, but it is now also used against other inflammatory bowel diseases (e.g ulcerative colitis). In the commercially available products (e.g. Remicade® or Inflectra®), infliximab is formulated as using sucrose, phosphate buffer and Tween 80 by freeze drying and given as intravenous infusion (Shire, 2015). The use of non-ionic Tween surfactants does raise concerns due to their propensity to autooxidation (causing oxidative damage to proteins or visible particle formation during long-term storage) (Wang et al., 2008; Dwivedi et al., 2018). In this present work, sugar and Tween were replaced by HP-β-CD as a promising excipient for stabilizing therapeutic proteins. Moreover, HP-β-CD as a non-polymeric matrix system is suitable for electrospun fiber formation due to its ability to provide highly viscous solution (Vass et al., 2019). According to the authors' best knowledge this is the first study reporting successful manufacture of a reconstitutable, electrospun mAb using pharmaceutical relevant scale electrospinning equipment. Different analytical methods evaluating the stability and biological activity of infliximab were carried out to investigate degradation during the various steps of the electrospinning process.

2. Materials and methods

2.1. Materials

Infliximab was provided by Janssen Pharmaceutica (Beerse, Belgium). In the reference liquid solution, infliximab was formulated in 10 mM sodium phosphate at a pH of 7.2 and at a protein concentration of 45 mg/mL. 2-Hydroxypropyl-beta-cyclodextrin (HP-β-CD) (Kleptose® HPB, MS nominal value: 0.62) was received from Roquette Pharma (Lestrem, France). Sodium-phosphate (Sigma-Aldrich, Budapest, Hungary) buffer was used to prepare the aqueous solution for electrospinning. The ultrapure water used in the experiments was from a Zeneer UP S-UV (Human Corporation) water system.

2.2. High-speed electrospinning (HSES)

The scaled-up electrospinning experiments were carried out using a HSES setup (Fig. 1), which consists of a disk-shaped stainless steel spinneret (diameter 34 mm) equipped with 36 orifices (diameter 500 μm), which is connected to a high-speed motor (Nagy et al., 2015). The electrospinning feed solution was prepared by gently mixing the HP-β-CD powder and the infliximab-containing (45 mg/ml) sodium phosphate buffer (10 mM, pH 7.2) using a shaking tray (100 rpm) at room temperature until complete dissolution. The feed solution was delivered to the system with a syringe pump (Harvard Apparatus Pump, Holliston, MA) using a flow rate of 100 mL/h. During the process the rotational speed of the spinneret was fixed at 40,000 rpm and the applied high voltage on it was 40 kV (power supply Unitronik Ltd., Nagykanizsa, Hungary). The experiments were performed at room temperature (25 °C) and at a relative humidity of 45 ± 5%. The produced fibers were collected in a cyclone. After the HSES process, the electrospun samples were stored at 2–5 °C before further analytical characterization.

2.3. Scanning electron microscopy (SEM)

The morphology of the samples was examined by means of a JEOL 6380LVa (Tokyo, Japan) type scanning electron microscope in high vacuum after electrospinning. Samples were placed on electrically conductive double-sided carbon adhesive tape and sputter-coated with gold (JEOL 1200, Tokyo, Japan). During the measurement, the accelerating voltage was set to 10 kV and the working distance was between 10 and 15 mm.

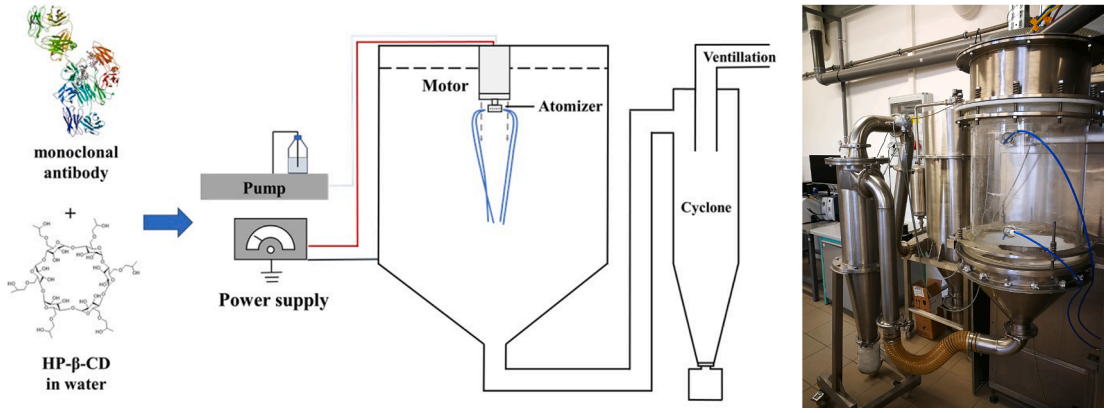

Fig. 1. High-speed electrospinning (HSES) device equipped with continuous cyclone sample collector.

2.4. Residual water content determination

Q5000 TGA (TA Instruments, New Castle, DE, USA) instrument was used to measure the residual water content based on the weight loss of approximately 10 mg sample. Sample was heated up from 25 to 105 °C by a rate of 10 °C/min and was kept at 105 °C for 10 min. The instrument was flushed with nitrogen gas at a flow rate of 25 mL/min during the measurement.

2.5. SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to examine the molecular mass of the different subunits, possible aggregates or smaller sized degradation products of infliximab. The measurement was carried out using a vertical Bio-Rad Mini-Protean 3 electrophoresis system (Bio-Rad Laboratories, Budapest, Hungary) under reducing and non-reducing conditions with 4–20% Mini-Protean TGX Precast Protein Gels (Bio-Rad Laboratories, Budapest). Samples were diluted in phosphate-buffered saline (PBS) to a concentration of 5–10 mg/mL, then 10 μL of each was mixed with 30 μL Laemmli sample buffer (4×) (Bio-Rad Laboratories, Budapest). For reduced samples, dithiothreitol (Sigma-Aldrich, Budapest, Hungary) was added to a final concentration of 50 mM. Samples were incubated for 10 min at 90 °C and after that 10 μL of each was loaded onto the gel. Precision Plus Protein Unstained Standards were used as a molecular weight ladder. Electrophoresis was performed at 100 V for approximately 50 min. After running, protein bands were visualized by staining the gel with Coomassie Brilliant Blue staining solution (Sigma-Aldrich, Budapest, Hungary) and destaining in the mixture of methanol, acetic acid, and water (40:10:50 v/v%).

2.6. Size exclusion chromatography

Size exclusion chromatography (SEC) was used to determine the percentage of infliximab aggregations or fragmentations. The measurements were performed using a Shimadzu HPLC system equipped with SPD-10A UV–Vis Detector. TSK-gel G3000SWXL column (Tosoh 7.8 mm × 30 cm, 5 μm) was used in combination with the mobile phase consisting of 0.2 M sodium phosphate buffer (pH 6.8). Protein samples were dissolved using the mobile phase and then filtered through a 0.45 μm filter (Millipore) before injection. 20 μL of the samples were injected onto the column and the separation was performed at a flow rate of 1 mL/min. The UV absorption was monitored at 280 nm. The size-based separation allowed creating a calibration curve based on a set of reference infliximab solution of known concentrations. This was then used to estimate the percentage of monomers, aggregates and fragments of infliximab in the unknown samples.

2.7. Bio-layer interferometry

The binding affinity of infliximab to the recombinant human Fc γ RIIIA/CD16a receptor (R&D Systems) was determined using the Octet K2 instrument (Pall ForteBio). The measurements were performed using Anti-Penta-HIS (HIS1K) Biosensor (Dip and Read™, ForteBio) tips at 30 °C with continuous agitation at 1000 rpm. The volume of the samples was 200 μL. The infliximab-containing electrospun sample was purified in advance by use of Amicon® Ultra-4 Centrifugal Filter Units with 10 kDa MWCO (Sigma Aldrich, Hungary) in order to eliminate the matrix effect of HP-β-CD. Kinetic assay was performed as follows: first, the HIS1K biosensor tip was equilibrated in PBS for 60 s to establish an initial baseline, then the HIS1K biosensor tip was loaded (200 s) into the solution of Fc γ RIIIA/CD16a receptor (diluted in PBS with 1% Tween 20). After that, a new baseline (30 s) was established, followed by the association (60 s) and dissociation (120 s) steps measured by dipping the receptor loaded biosensor into the wells containing the PBS solution of infliximab, and PBS with 1% Tween 20 buffer respectively. The biosensor tips were regenerated with 0.1 M citrate buffer (pH 3.5) after each assay cycle. To determine the dissociation constant (K_D), eight concentration settings (5; 2.5; 1.25; 0.625; 0.313; 0.156; 0.078, 0.039 μM) of infliximab were evaluated at constant receptor loading (1.5 μM). The generated data were evaluated by a globally fitted 1:1 binding interaction model using the Data Analysis HT 10.0 software.

2.8. Reconstitution test

A small-scale reconstitution test of the infliximab-containing electrospun product was carried out to investigate the reconstitution property of the nanofibrous sample. In this small-scale reconstitution test 0.53 g of the electrospun sample (containing 10 mg infliximab) was weighed and 1 mL ultrapure water was pipetted into a glass vial to obtain a theoretical concentration of 10 mg/mL. After water addition, the vial was gently swirled to evenly distribute the solvent and dissolve all the fibrous material. In order to evaluate the time of reconstitution and the appearance of the prepared solution, the test was monitored by video recording.

2.9. Viscosity measurement

AR 2000 rotational rheometer (TA Instruments, New Castle, USA) equipped with a parallel plate configuration was applied to determine the viscosity of the reconstituted solution. The diameter of the upper moving plate was 40 mm. Peltier plate was used to maintain the solution temperature at 25 °C. Both plates were made of stainless steel andthe applied gap between them was 500 μm. The viscosities were measured at shear rate of 50 s− (Shire, 2015). The reported viscosity is the average of 3 parallel measurements.

Table 1
Details of HSES experiments of the infliximab containing HP-β-CD matrix systems.

Applied conditions				Composition of matrix solution			Results of HSES		
Feeding rate [ml/h]	High voltage [kV]	Rotational speed [rpm]	Temperature [°C]	Buffer [g]	infliximab [mg]	HP-β-CD [w/w%]	Fiber diameter [μm]	Residual water content of the fibers [w/w %]	Yield [w/w %]
100	40	40,000	25	18	810	62.2	2.5 ± 0.9	6.4 ± 1.1	80 ± 5

3. Results and discussion

3.1. High-speed electrospinning (HSES) of infliximab

In this research, HSES was applied to investigate the feasibility of producing electrospun mAb on a relevant industrial (small) scale. According to the results of our previous study (Vass et al., 2019), 67.4 w/w % is the ideal concentration for HP-β-CD-based matrix solution. Such a high concentration is required because it promotes CD molecules to form strong and large polymer-like supramolecular structures in the solution enabling fiber formation during ES (Celebioglu and Uyar, 2012; Balogh et al., 2015). In the infliximab HSES experiment, the addition of mAb-containing sodium phosphate buffer as a solvent promoted the electrospun fiber formation, thus the aqueous matrix solution of 62.2 w/w% was applied under the applied circumstances (rpm, electric field and flow rate). The HSES was successfully performed at room temperature, the results of the experiment are summarized in Table 1.

Fig. 2 shows the morphology of the placebo CD and the mAb + CD electrospun fibers. As can be seen, infliximab did not have significant impact on fiber formation, and the sample has uniform and bead-free fibrous structure with an average fiber diameter of 2.47 ± 0.89 μm. It is also noticeable, that some fibers were broken into shorter fragments, which could be due to the circular motion of the fibers in the collector bin caused by the centrifugal forces inside the cyclone. The residual water content (measured thermogravimetrically) was 6.4 ± 1.1%, which is the equilibrium water content of the HP-β-CD (Vass et al., 2019). This is a higher value compared to the commercially available parenteral drug powders, however there are several ways that can be applied to decrease the residual water content of the HP-β-CD fibers, such as decreasing the relative humidity of the drying air during production or using a post-drying step. During the process the feeding rate was set to 100 mL/h, which equals to the productivity rate of ~72 g/h calculated from solution density of 1.126 g/cm^3. The monoclonal antibody content of the fibers was 18.8 mg infliximab/1 g fiber. The yield of the process was 80 ± 5% which could continue to improve by prolonged production and by further process and equipment optimizations. Theoretically, for the solid form of infliximab, a productivity of ~120,000 vials per year (each containing 100 mg infliximab/vial) is attainable with the current HSES setup when operated in continuous mode.

3.2. Physical stability of infliximab in the HP-β-CD electrospun material

Size exclusion chromatography (SEC) was used to detect any possibly occurring small size aggregates (e.g dimer, trimer) or fragments in the samples. The relative amount of the different forms of infliximab was calculated from the area under the UV peak at 280 nm. The results of the measurements are summarized in Table 2.

In the reference liquid formulation of infliximab, the main peak was eluted at retention time (RT) of 8.400 min and besides that no extra peak was observed. Accordingly, the reference sample represented an infliximab monomer content of 100% free of aggregates and fragments. The infliximab containing HP-β-CD aqueous solution and the reconstituted fibrous sample were also measured. In the aqueous solution, the main peak (RT = 8.398 min) corresponding to the monomeric form represented 99.25% of total AUC. In addition, there was a small peak at RT of 7.208 min indicating the presence of a few units with higher hydrodynamic diameter. The formation of small amount of aggregates can be attributed to the fact that during sample preparation the infliximab containing aqueous solution was stored and gently shaken at room temperature until complete dissolution. Accordingly, the solution preparation can be optimized (e.g using lower temperature condition and an efficient procedure for mixing infliximab with HP-β-CD) to minimize aggregation. In the chromatogram representing the electrospun formulation (Fig. 3) only the peaks of low molecule weight compounds (HP-β-CD and the eluent citrate) were observed at RT around 12 min beyond the elution of the aggregated and non-aggregated forms of infliximab. After the HSES process the detected aggregates increased only by 0.01 percentage points (compared to the initial aqueous HP-β-CD solution), which is comparable to the standard deviation of the measurement. Overall, this shows that electrospinning was a viable alternative drying technique to freeze drying as no significant reduction was observed for the monomer content.

SDS-PAGE was performed to reveal the presence of degradation products that may have formed during the electrospinning process. Fig. 4 displays the images of the gel electrophoresis. Under non-reducing conditions, the position of the major bands was similar in each sample at about 150 kDa, which corresponds to the monomeric form of infliximab. In the samples of the aqueous matrix solution and the solid electrospun formulation, no signs of aggregates or lower molecular weight regions were detected. Under reducing conditions, all samples showed two intensive bands at about 50 and 25 kDa deriving from the antibody fragments (heavy and light chain) formed by the disulfide bonds cleavage. Additional bands at 75 kD were found in all the three samples, suggesting the formation of half molecules (a combination of one heavy and one light chain). The position of the fragments was identical to that of the fragment obtained in the infliximab reference solution. According to these analytical results, the rapid drying provided by high-speed electrospinning process did not induce significant antibody aggregation or fragmentation.

3.3. Binding affinity

The biological activity of infliximab after high-speed electrospinning was assessed by measuring its binding ability to Fc γ RIIIA/CD16a receptor. The kinetic assay was performed using Bio-Layer Interferometry (BLI), which is a powerful optical technique enabling real-time direct measurement of macromolecular interactions without the need for labeled reagents. In order to examine the effect of high-speed electrospinning on the biological activity of infliximab, the BLI sensors with a thin immobilized bio-layer on the surface at the tips were dipped into the samples of ES and reference liquid formulations on a 96-well microplate. Due to the tip surface-bound molecules, the increased thickness of the molecular layer was analyzed by measuring the interference pattern between waves of white light reflected from the surfaces at the detector. In the case of the electrospun sample, it was necessary to filter the material from HP-β-CD as the complexing ability of CD can influence the binding of infliximab to the receptor. Fig. 5 represents the kinetic sensorgram of infliximab after the HSES process. It can be divided into three main parts: the baseline step (0–30 s), and the steps of the association (30–90 s) and dissociation (90–200 s) of receptor-antibody complex. The binding pattern of infliximab in the electrospun sample was very similar to the reference infliximab solution (data not shown). The blue curves on the sensorgram are corresponding to the kinetic assay of infliximab at each adjusted analyte concentration. The

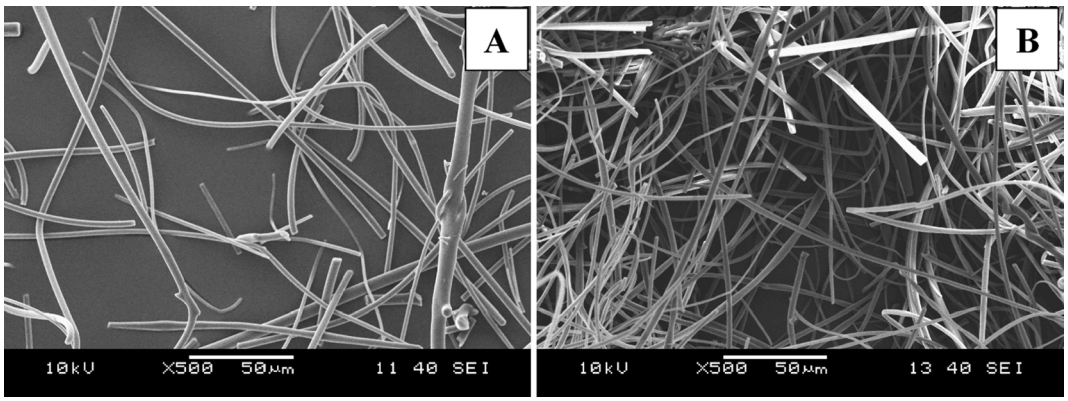

Fig. 2. Scanning electron microscopic images of the placebo HP-β-CD electrospun sample (A) and infliximab-containing HP-β-CD electrospun sample (B).

red curves were fitted and analyzed globally by the software based on the 1:1 binding model.

Based on the data analyzed from these sensorgrams, the kinetic parameters were determined. The individual equilibrium binding constants (K_D) (calculated as k_d/k_a ratio, where k_a and k_d are the association dissociation ratios, respectively), the standard errors (SE) and the model fitting parameters (R^2 - the coefficient of determination and χ^2 - the sum of the squared deviation) of the two samples are summarized in Table 3. The quality of the curve fitting is acceptable if the total R^2 is above 0.95 and the total χ^2 is below 3 (Tobias and Kumaraswamy, 2014). The calculated binding constants are reliable, the standard errors do not exceed ±10%. According to the results, there was no remarkable difference in binding affinity before (reference sample) and after the HSES process (electrospun sample purified from HP-β-CD), since the Kd values were 3.69 ± 0.06 10^{-7} M and 3.68 ± 0.07·10^{-7}, respectively. This means, that HSES process did not cause a significant change in binding affinity of infliximab.

Table 2
Results of size exclusion chromatography.

Sample	Main peak		Side peaks	
	Retention time [min]	Monomer [%]	Retention time [min]	Aggregation [%]
infliximab reference liquid formulation	8.400	100	–	0
infliximab HP-β-CD aqueous solution	8.398	99.25	7.208	0.75
infliximab HP-β-CD electrospun formulation	8.392	99.24	7.167	0.76

3.4. Reconstitution test

In the case of freeze dried powders (especially for high-concentration formulations), it is a unique challenge to provide fast reconstitution capabilities prior to parenteral administration (Luoma and Lim, 2020). The application of ES technology could be beneficial to improve the dissolution properties from the enhanced surface area of the produced fibers. A small-scale reconstitution test was performed to investigate the dissolution properties of the electrospun material. Following the preparation and administration instructions of Remicade®, the test requires a vial containing 100 mg of infliximab and 10 mL of sterile water for

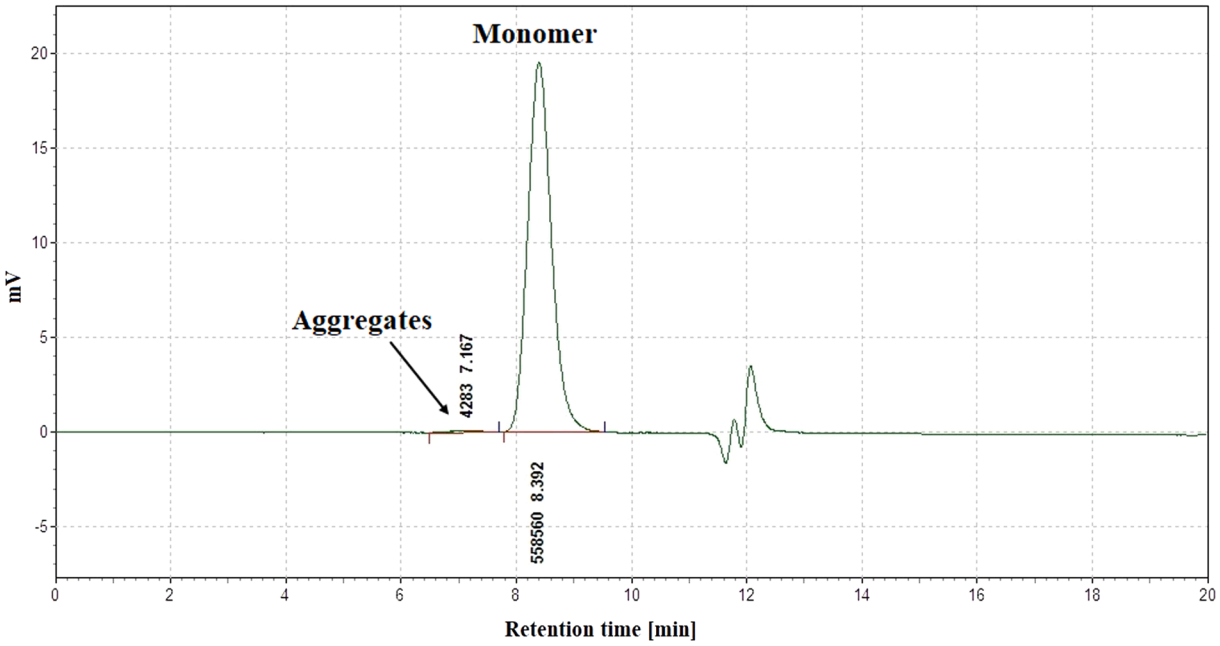

Fig. 3. Size exclusion chromatogram of infliximab-based electrospun sample.

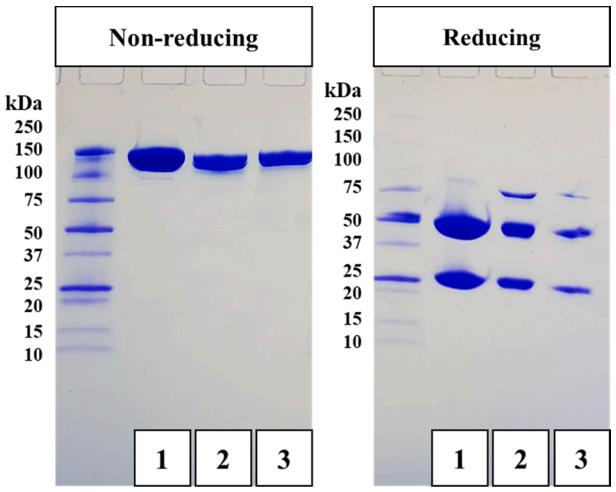

Fig. 4. Gel images of SDS-PAGE under non-reduced (left) and reduced (right) conditions; (1) reference liquid formulation of infliximab; (2) aqueous HP-β-CD solution of infliximab; (3) electrospun sample of infliximab.

injection (U.S. Food and Drug Administration (FDA), 2020). In our case, this is equivalent to 5.32 g HP-β-CD-based fibrous sample. In this small-scale test, 1 mL of pure water was added into a vial containing 0.53 g of electrospun material (containing 10 mg infliximab). Consequently, the resulting concentration of the final solution was 10 mg/mL for infliximab. As it can be seen on the recorded images, the sample dissolved completely within 2 min without any vigorous mixing (Fig. 6).

Owing to the large surface area of the HP-β-CD fibers, the dried product had excellent dissolution properties achieving a clear and homogenous solution. The reconstituted sample was visually inspected, the solution was limpid and no visible particles were observed after 5 min. For optimal parenteral drug delivery, the viscosity is one of the critical properties as it greatly determines the injectability of the liquid formulation. The viscosity of the reconstituted solution was found to be 2.96 ± 0.35 mPa·s, which is suitable for intravenous infusion application as the recommended maximum viscosity is 40 mPa·s (Wilson, 2007).

4. Conclusion

The aim of this study was to evaluate high-speed electrospinning as an alternative to currently prevalent freeze drying for the production of a reconstitution dosage form of infliximab. The HSES process was successfully carried out at room temperature from infliximab-containing HP-β-CD aqueous solution resulting homogenous and bead-free fiber formation. During the process the applied feeding rate was 100 mL/h, equaling a productivity of ~72 g/h, which can be further improved if the HSES equipment is operated for a longer period. The produced fibers were collected by a cyclone with 80% yield in which 18.8 mg mAb/1 g product was achieved. Based on the results of the analytical measurements (size exclusion chromatography and SDS-PAGE electrophoresis), high-speed electrospinning did not cause degradation of infliximab, only a negligible sign of aggregation and no fragmentation could be detected in the initial aqueous matrix solution. The Bio-layer Interferometry measurements demonstrated that the kinetic parameters of infliximab did not change after the ES process compared to the reference liquid formulation. Accordingly, HSES had no negative influence on the binding affinity of infliximab to Fc γ receptor. The small-scale reconstitution test demonstrated that the infliximab-containing electrospun material can be dissolved within 2 min, which is fully acceptable for a reconstitution dosage form. Overall, it can be concluded that high-speed electrospinning proved to be a suitable technology for the manufacture of a solid formulation of a monoclonal antibody. Furthermore, these results indicated that HP-β-CD is a very promising agent for fiber formation as well as for stabilizing therapeutic antibodies in solid electrospun formulations. Further process optimization and long-term stability tests are foreseen in the next stage of the research work.

CRediT authorship contribution statement

Júlia Domján: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Panna Vass:** Conceptualization, Investigation, Formal analysis, Writing - review & editing. **Edit Hirsch:** Conceptualization, Investigation, Formal analysis, Writing - review & editing. **Edina Szabó:** Visualization, Investigation. **Eszter Pantea:** Visualization, Investigation. **Sune K. Andersen:** Writing - review & editing. **Tamás Vigh:** Writing - review & editing. **Geert Verreck:** Writing - review & editing. **György Marosi:** Writing - review & editing, Funding

Table 3
Results of kinetic rate constants for binding to Fc γ RIIIA/CD16a by infliximab.

Sample	K_D ± SE[M]	R^2	χ^2
infliximab liquid formulation	3.69 ± 0.06E-07	0.989	1.03
infliximab electrospun formulation	3.68 ± 0.07E-07	0.988	1.98

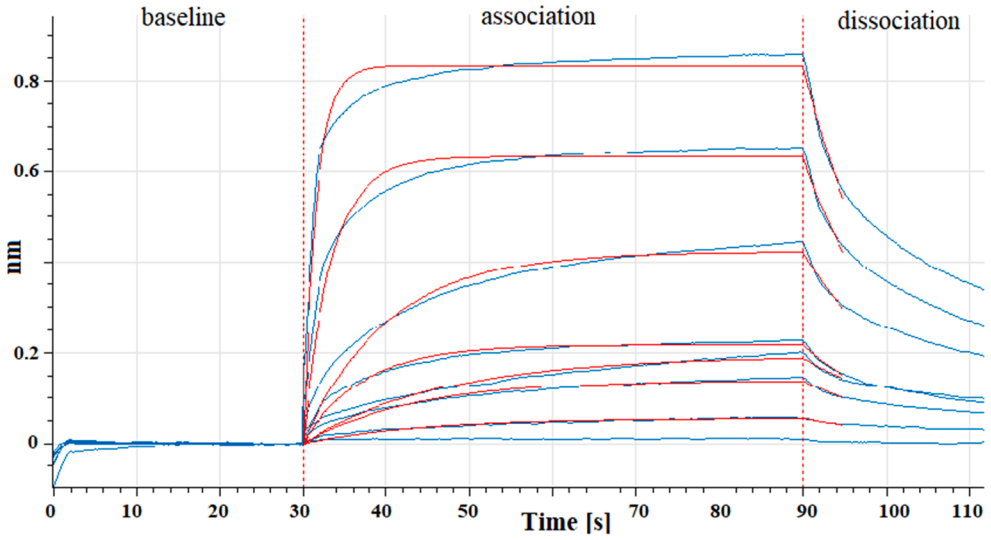

Fig. 5. Binding sensorgram of infliximab after high-speed electrospinning.

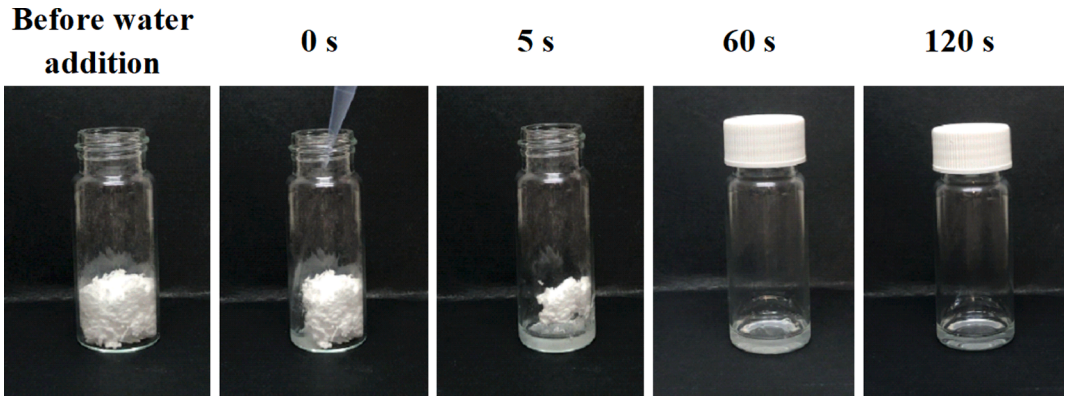

Fig. 6. Small-scale dissolution test of infliximab-based electrospun sample.

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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.

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References

Abdul-Fattah, A.M., Truong, V.L., 2010. Drying process methods for biopharmaceutical products: an overview. Formul. Process Dev. Strateg. Manuf. Biopharm. 705–738. https://doi.org/10.1002/9780470595886.ch28.

Angkawinitwong, U., Sharma, G., Brocchini, S., 2015. Solid-state protein formulations. Ther. Deliv. 6, 59–82. https://doi.org/10.4155/tde.14.98.

Angkawinitwong, U., Awwad, S., Khaw, P.T., Brocchini, S., Williams, G.R., 2017. Electrospun formulations of bevacizumab for sustained release in the eye. Acta Biomater. 64, 126–136. https://doi.org/10.1016/j.actbio.2017.10.015.

Balogh, A., Horváthová, T., Fülöp, Z., Loftsson, T., Harasztos, A.H., Marosi, G., Nagy, Z. K., 2015. Electroblowing and electrospinning of fi brous diclofenac sodium-cyclodextrin complex-based reconstitution injection. J. Drug Deliv. Sci. Technol. 26, 28–34. https://doi.org/10.1016/j.jddst.2015.02.003.

Batens, M., Massant, J., Teodorescu, B., Van den Mooter, G., 2018. Formulating monoclonal antibodies as powders for reconstitution at high concentration using spray drying: models and pitfalls. Eur. J. Pharm. Biopharm. 127, 407–422. https://doi.org/10.1016/j.ejpb.2018.02.002.

Bowen, M., Turok, R., Maa, Y., 2013. Spray drying of monoclonal antibodies: investigating powder-based biologic drug substance bulk storage spray drying of monoclonal antibodies: investigating powder-based biologic drug substance bulk storage. Dry. Technol. 31 (13–14), 1441–1450. https://doi.org/10.1080/07373937.2013.796968.

Branchu, S., Forbes, R.T., York, P., Petren, S., Nyqvist, H., Camber, O., 1999. Hydroxypropyl-β-cyclodextrin inhibits spray-drying-induced inactivation of β-galactosidase. J. Pharm. Sci. 88, 905–911. https://doi.org/10.1021/js9804819.

Capozzi, L.C., Trout, B.L., Pisano, R., 2019. From batch to continuous: freeze-drying of suspended vials for pharmaceuticals in unit-doses. Ind. Eng. Chem. Res. 58 (4), 1635–1649. https://doi.org/10.1021/acs.iecr.8b02886.

Celebioglu, A., Uyar, T., 2012. Electrospinning of nanofibers from non-polymeric systems: polymer-free nanofibers from cyclodextrin derivatives. Nanoscale 4 (2), 621–631. https://doi.org/10.1039/c1nr11364j.

Chang, B.S., Hershenson, S., 2002. Practical approaches to protein formulation development. Ration. Des. Stable Protein Formul. 13, 1–25. https://doi.org/10.1007/978-1-4615-0557-0_1.

Chang, L.L., Shepherd, D., Sun, J., Ouellette, D., Grant, K.L., Tang, X.C., Pikal, M.J., 2005. Mechanism of protein stabilization by sugars during freeze-drying and storage: native structure preservation, specific interaction, and / or immobilization in a glassy matrix? J. Pharm. Sci. 94 (7), 1427–1444. https://doi.org/10.1002/jps.20364.

Choi, J.S., Han, S., Hyun, C., Yoo, H.S., 2015. Buccal adhesive nanofibers containing human growth hormone for oral mucositis. J. Biomed. Mater. Res. Part B 104, 1396–1406. https://doi.org/10.1002/jbm.b.33487.

Cordoba-Rodriguez, R.V., 2008. Aggregates in MAbs and recombinant therapeutic proteins: a regulatory perspective. BioPharm Int. 21 (11), 44–53.

Daugherty, A.L., Mrsny, R.J., 2006. Formulation and delivery issues for monoclonal antibody therapeutics. Adv. Drug Deliv. Rev. 58 (5–6), 686–706. https://doi.org/10.1016/j.addr.2006.03.011.

Davis, M.E., Brewster, M.E., 2004. Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. Drug Discov. 3 (December), 1023–1035. https://doi.org/10.1038/nrd1576.

De Meyer, L., Van Bockstal, P.J., Corver, J., Vervaet, C., Remon, J.P., De Beer, T., 2015. Evaluation of spin freezing versus conventional freezing as part of a continuous pharmaceutical freeze-drying concept for unit doses. Int. J. Pharm. 496 (1), 75–85. https://doi.org/10.1016/j.ijpharm.2015.05.025.

Dwivedi, M., Blech, M., Presser, I., Garidel, P., 2018. Polysorbate degradation in biotherapeutic formulations: identification and discussion of current root causes. Int. J. Pharm. 552, 422–436. https://doi.org/10.1016/j.ijpharm.2018.10.008.

Emami, F., Vatanara, A., Park, E.J., Na, D.H., 2018. Drying technologies for the stability and bioavailability of biopharmaceuticals. Pharmaceutics 10 (3), 1–22. https://doi.org/10.3390/pharmaceutics10030131.

Faghihi, H., Khalili, F., Amini, M., Vatanara, A., Faghihi, H., Khalili, F., Amini, M., Vatanara, A., 2015. The effect of freeze-dried antibody concentrations on its stability in the presence of trehalose and hydroxypropyl-β-cyclodextrin: a box–. Behnken Statistical Design. 7450, 724–732. https://doi.org/10.3109/10837450.2015.1116563.

Greiner, A., Wendorff, J.H., 2007. Electrospinning : a fascinating method for the preparation of ultrathin fibers angewandte. 46 (30), 5670–5703. 10.1002/anie.200604646.

Haeuser, C., Goldbach, P., Huwyler, J., Friess, W., Allmendinger, A., 2020. Excipients for room temperature stable freeze-dried monoclonal antibody formulations. J. Pharm. Sci. 109 (1), 807–817. https://doi.org/10.1016/j.xphs.2019.10.016.

Harn, N., Spitznagel, T., Perkins, M., Allan, C., Shire, S., Middaugh, C.R., 2010. Current Trends in Monoclonal Antibody Development and Manufacturing. Springer, New York, 10.1007/978-0-387-76643-0.

Hirsch, E., Vass, P., Démuth, B., Pethő, Z., Bitay, E., Andersen, S.K., Vigh, T., Verreck, G., Molnár, K., Nagy, Z.K., Marosi, G., 2019. Electrospinning scale-up and formulation development of PVA nanofibers aiming oral delivery of biopharmaceuticals. Express Polym. Lett. 13 (7), 590–603. https://doi.org/10.3144/expresspolymlett.2019.50.

Hu, X., Liu, S., Zhou, G., Huang, Y., Xie, Z., Jing, X., 2014. Electrospinning of polymeric nanofibers for drug delivery applications. J. Control. Release 185, 12–21. https://doi.org/10.1016/j.jconrel.2014.04.018.

Huang, Z., Zhang, Y., Kotaki, M., Ramakrishna, S.A., 2003. Review on polymer nanofibers by electrospinning and their applications in nanocomposites. 63 (15), 2223–2253. 10.1016/S0266-3538(03)00178-7.

Iwai, J., Ogawa, N., Nagase, H., Endo, T., Loftsson, T., 2007. Effects of various cyclodextrins on the stability of freeze-dried lactate dehydrogenase. 96 (11), 3140–3143. 10.1002/jps.20847.

Jiang, H., Wang, L., Zhu, K., 2014. Coaxial electrospinning for encapsulation and controlled release of fragile water-soluble bioactive agents. J. Control. Release 193, 296–303. https://doi.org/10.1016/j.jconrel.2014.04.025.

Li, B.D., Xia, Y., 2004. Electrospinning of nanofibers: reinventing the wheel? Adv. Mater. 16 (14), 1151–1170. https://doi.org/10.1002/adma.200400719.

Lowe, D., Dudgeon, K., Rouet, R., Schofield, P., Jermutus, L., Christ, D., 2011. Aggregation, stability, and formulation of human antibody therapeutics. Adv. Protein Chem. Struct. Biol. 84, 41–61. https://doi.org/10.1016/B978-0-12-386483-3.00004-5.

Luoma, J., Lim, F.J., 2020. Strategies to reduce reconstitution time of lyophilized biotherapeutics. J. Pharm. Sci. 109 (6), 1905–1911. https://doi.org/10.1016/j.xphs.2020.02.019.

Nagy, Z.K., Wagner, I., Suhajda, Á., Tobak, T., Harasztos, A.H., Vigh, T., Sóti, P.L., Pataki, H., Molnár, K., Marosi, G., 2014. Nanofibrous solid dosage form of living bacteria prepared by electrospinning. Express Polym. Lett. 8 (5), 352–361. https://doi.org/10.3144/expresspolymlett.2014.39.

Nagy, Z.K., Balogh, A., Démuth, B., Pataki, H., Vigh, T., Szabó, B., Molnár, K., Schmidt, B. T., Horák, P., Marosi, G., Verreck, G., Van Assche, I., Brewster, M.E., 2015. High speed electrospinning for scaled-up production of amorphous solid dispersion of itraconazole. Int. J. Pharm. 480 (1–2), 137–142. https://doi.org/10.1016/j.ijpharm.2015.01.025.

Otzen, D.E., Knudsen, B.R., Aachmann, F., Larsen, K.I.M.L., Wimmer, R., 2002. Structural basis for cyclodextrins' suppression of human growth hormone aggregation. 1779–1787. 10.1110/ps.0202702.philic.

Ramezani, V., Vatanara, A., Seyedabadi, M., Nabi Meibodi, M., Fanaei, H., 2017. Application of cyclodextrins in antibody microparticles: potentials for antibody protection in spray drying. Drug Dev. Ind. Pharm. 43 (7), 1103–1111. https://doi.org/10.1080/03639045.2017.1293679.

Rosenberg, A.S., 2006. Effects of protein aggregates: an immunologic perspective. AAPS J. 8 (3), 501–507. https://doi.org/10.1208/aapsj080359.

Serno, T.I.M., Carpenter, J.F., Randolph, T.W., Winter, G., 2010. Inhibition of agitation-induced aggregation of an IgG-antibody by hydroxypropyl- b -cyclodextrin. 99 (3), 1193–1206. 10.1002/jps.21931.

Serno, T., Geidobler, R., Winter, G., 2011. Protein stabilization by cyclodextrins in the liquid and dried state. Adv. Drug Deliv. Rev. 63 (13), 1086–1106. https://doi.org/10.1016/j.addr.2011.08.003.

Shire, S.J., 2015. Formulation of proteins and monoclonal antibodies (MAbs). In: Monoclonal Antibodies. Woodhead Publishing, pp. 93–120. https://doi.org/10.1016/B978-0-08-100296-4.00004-X.

Tobias, R., Kumaraswamy, S., 2014. Biomolecular binding kinetic assays on the octet platform. Forte Bio. Appl. Note 14, 1–21.

Tyo, K.M., Duan, J., Kollipara, P., Vincent, M., Cerna, C., Lee, D., Palmer, K.E., Steinbach-rankins, J.M., 2019. PH-responsive delivery of Griffithsin from electrospun fibres. Eur. J. Pharm. Biopharm. 138, 64–74. https://doi.org/10.1016/j.ejpb.2018.04.013.

U.S. Food and Drug Administration (FDA), 2020. Remicade (infliximab) label. https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/%0A103772s5359lbl.pdf (accessed Mar 25, 2020).

Uchiyama, S., 2014. Liquid formulation for antibody drugs. Biochim. Biophys. Acta - Proteins Proteomics 1844 (11), 2041–2052. https://doi.org/10.1016/j.bbapap.2014.07.016.

Vass, P., Démuth, B., Hirsch, E., Nagy, B., Andersen, S.K., Vigh, T., 2019. Drying technology strategies for colon-targeted oral delivery of biopharmaceuticals. J. Control. Release 296, 162–178. https://doi.org/10.1016/j.jconrel.2019.01.023.

Vass, P., Nagy, Z.K., Kóczián, R., Fehér, C., Démuth, B., Szabó, E., Andersen, S.K., Vigh, T., Verreck, G., Csontos, I., Marosi, G., Hirsch, E., 2019. Continuous drying of a protein-type drug using scaled-up fiber formation with HP- β -CD matrix resulting in a directly compressible powder for tableting. Eur. J. Pharm. Sci. 141, 105089 https://doi.org/10.1016/j.ejps.2019.105089.

Vass, P., Démuth, B., Farkas, A., Hirsch, E., Szabó, E., Nagy, B., Andersen, S.K., Vigh, T., Verreck, G., Csontos, I., Marosi, G., Nagy, Z.K., 2019. Continuous alternative to freeze drying: manufacturing of cyclodextrin- based reconstitution powder from aqueous solution using scaled-up electrospinning. J. Control. Release 298, 120–127. https://doi.org/10.1016/j.jconrel.2019.02.019.

Wagner, I., Nagy, Z.K., Vass, P., Fehér, C., Barta, Z., Vigh, T., Sóti, P.L., Harasztos, A.H., Pataki, H., Balogh, A., Verreck, G., Assche, I.V., Marosi, G., 2015. Stable formulation of protein-type drug in electrospun polymeric fiber followed by tableting and scaling-up experiments. Polym. Adv. Technol. 26 (12), 1461–1467. https://doi.org/10.1002/pat.3569.

Wang, W., 2000. Lyophilization and development of solid protein pharmaceuticals. Int. J. Pharm. 203 (1–2), 1–60. https://doi.org/10.1016/S0378-5173(00)00423-3.

Wang, W., Wang, Y.J., Wang, D.Q., 2008. Dual effects of tween 80 on protein stability. 347, 31–38. 10.1016/j.ijpharm.2007.06.042.

Wilson, W.C., 2007. Trauma: Emergency Resuscitation Perioperative Anesthesia Surgical Management Vol. 1, 197–213.

Zussman, E., 2011. Encapsulation of cells within electrospun fibers. Polym. Adv. Technol. 22 (3), 366–371. https://doi.org/10.1002/pat.1812.