*proceedings*

MDPI

*Proceedings*

# Optimization of the Emulsion Electrospinning for Increased Activity of Biopharmaceuticals †

**Natalia Burlaga [1,2], Magdalena Bartolewska [2,3], Matej Buzgo [2] and Aiva Simaite [2,*]**

[1] Institute of Chemical Technology and Engineering, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland; natalia.burlaga@doctorate.put.poznan.pl
[2] InoCure s.r.o., R&D Lab, Prumyslová 1960, 250 88 Celákovice, Czech Republic; bartolewskamagdalena@gmail.com (M.B.); matej@inocure.cz (M.B.)
[3] Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
* Correspondence: aiva@inocure.cz; Tel.: +420-(775)-876536
† Presented at the 1st International Electronic Conference on Pharmaceutics, 1–15 December 2020; Available online: https://iecp2020.sciforum.net/.

**Abstract:** High-throughput emulsion electrospinning is a technology that can enable practical nanofiber application for drug delivery. Core-shell structure of the electrospun fibers allows the encapsulation of the active pharmaceutical ingredients (APIs), protects their activity, and controls their release rate. However, electrospinning using high flow rates usually requires high electric fields that may negatively affect the activity of the biomolecules. Moreover, charged APIs tend to migrate to the surface of the fibers during the electrospinning process leading to the high burst release. That is disadvantageous when long-term sustained release is needed. We have investigated the influence of the electrospinning parameters such as distances between the electrode and collector and the applied voltages to both activity of the encapsulated proteins and their burst release. We have also tested the influence of number of the stabilizers, e.g., trehalose, pluronic, and polyvinylpyrrolidone, on their ability to preserve the protein activity, and the influence of the different molecular weights of polyvinyl alcohol on the ability to sustain the release. Our results demonstrate the importance of the water phase composition to both activity and release and are critical for further understanding of the processes taking place during the emulsion electrospinning.

**Keywords:** high-throughput electrospinning; emulsion electrospinning; biopharmaceuticals; protein activity; drug release

**Citation:** Burlaga, N.; Bartolewska, M.; Buzgo, M.; Simaite, A. Optimization of the Emulsion Electrospinning for Increased Activity of Biopharmaceuticals. *Proceedings* **2021**, *78*, 39. https://doi.org/10.3390/IECP2020-08673

Published: 1 December 2020



## 1. Introduction

Peptides and proteins are used as therapeutics for various diseases such as diabetes, cancer, infections, and autoimmune disorders among others. To protect and effectively deliver sensitive protein based active pharmaceutical ingredients (APIs) to their target, various design, formulation, and administration strategies are considered. The core/shell structures are promising tools for increasing the stability of biomolecule-based APIs. With suitable shell polymer, drugs encapsulated in the core can be protected from humidity, light, heat, and oxygen [1]. Core-shell electrospinning is one of the methods to safely incorporate therapeutic proteins to the nanofibrous scaffolds that can then be used for API's sustained release at the diseased site [2]. However, practical applications of the most common method for core-shell fiber preparation-coaxial electrospining are limited by the extremely low production throughput (<0.5 mL $h^{-1}$). Emulsion electrospinning is an alternative technique which can be easily up-scaled using the needle-less electrodes [3]. However, high-throughput electrospinning often requires high electric fields that may cause loss of activity of protein-based therapeutics.

During the electrospinning of the water-in-oil emulsions, the organic solvent evaporates faster than water causing the water phase droplets to travel inwards and to be stretched to form fibers with a continuous hydrophilic core [4,5]. Many factors may influence proteins' activity during electrospinning [6–8]. First of all, protein activity may be affected by the composition of the water phase. It was previously reported that certain surfactant may cause protein denaturation [9]. A protein's structure may also be affected by the interface with the organic solvent [10,11]. Moreover, while emulsification by high speed homogenizers is needed to create smaller droplets and stabilize the emulsion, the process may as well affect protein activity [12–14]. Finally, high-throughput electrospinning often requires higher electric fields (up to 90 kV) and exposure of proteins to high electric field may also lead to their denaturation [3].

In this work, we investigated multiple factors that may influence protein during the high-throughput emulsion electrospinning process. More specifically, we have tested the impact of stabilizers (i.e., trehalose, pluronic, polyvinylpyrrolidone), different molecular weights of polyvinyl alcohol (PVA), and the influence of the applied voltage on activity of the encapsulated proteins. In order to provide a balanced overview of the optimum electrospinning parameters, fiber morphology and the protein burst release were also characterized.

## 2. Experiments

### *2.1. Materials*

Polymers and surfactants: 45 kDa polycaprolactone (PCL) from Sigma-Aldrich; poly(vinyl alcohol) (PVA) 5-88 and 40-88 from Emprove Merck and PVA 26-88, 28-99, 56-98 and 4-99 from Sigma-Aldrich; 8400 Da Pluronic F-68 (PF68) from PanReac AppliChem; 3300 Da Pluronic 31R1 (P31R1) and polyvinylpyrrolidone K-30 (PVP) from Sigma-Aldrich; D-(+)-trehalose dihydrate 99% from Alfa Aesar. Solvents: chloroform stabilized with 0.6% ethanol, ethanol absolute 99.7% and technical water were purchased from VWR International. Horseradish peroxidase (HRP) was purchased from PanReac AppliChem. Protein release and activity testing: Micro BCA™ Protein Assay Kit and 1-Step™ Turbo TMB-ELISA Substrate Solution were purchased from Thermo Fisher Scientific; sulfuric acid 95–98% was purchased from Emprove Merck.

### *2.2. Methods*

#### 2.2.1. Emulsion Preparation for Electrospinning Process

For electrospinning experiments, all solutions were prepared by magnetic stirring. The following stocks were made: 36% PCL in chloroform:ethanol in ratio 9:1, 5% (*w*/*v*) PF68/PVP/trehalose, 15% (*w*/*v*) PVAs and 10 mg $mL^{-1}$ HRP in distilled water (DW). The concentrated stock solutions were diluted to the needed final concentration just before the electrospinning process. Further on, all concentrations are provided per volume of the respective emulsion phase and not as a total concentration in the solution. For all experiments, water-in-oil (*w*/*o*) emulsions were used with 32% of PCL and 0.02% of P31R1 in chloroform:ethanol (9:1) as an oil phase (OP). P31R1 was added to an OP as a low HLB index surfactant. The water phase (WP) contained PF-68/PVP/trehalose, PVA, and HRP dissolved in DW and mixed in concentrations listed in Table 1. In the first two experiments (1 and 2), the mixtures of organic and water phases were homogenized for 2 min with the speed 6600 rpm using IKA T-18 Digital ULTRA TURRAX. In experiment 3, emulsions were mixed by shaking by hand. In all formulations, the OP:WP ratio was 9:1.

**Table 1.** Variable parameters in individual experiments: the composition of the emulsion used in the electrospinning process, applied voltage and the method of mixing the emulsion.

| Exp. | Sample | Composition of Water Phase | Voltage (–/+) kV | Emulsion Mixing |
|---|---|---|---|---|
| 1 | 1A | 3% 5–88 kDa polyvinyl alcohol (PVA) + 6% PF68 + 0.2% Horseradish peroxidase (HRP) + distilled water (DW) | 30/40 | by homoge-nizer |
| | 1B | 3% 5–88 kDa PVA + 6% PVP + 0.2% HRP + DW | | |
| | 1C | 3% 5–88 kDa PVA + 6% trehalose + 0.2% HRP + DW | | |
| 2 | 2A | 3% 4–99 PVA + 6% trehalose + 0.2% HRP + DW | 30/40 | by homoge-nizer |
| | 2B | 3% 28–99 PVA + 6% trehalose + 0.2% HRP + DW | | |
| | 2C | 3% 56–98 PVA + 6% trehalose + 0.2% HRP + DW | | |
| | 2D | 3% 5–88 PVA + 6% trehalose + 0.2% HRP + DW | | |
| | 2E | 3% 26–88 PVA + 6% trehalose + 0.2% HRP + DW | | |
| | 2F | 3% 40–88 PVA + 6% trehalose + 0.2% HRP + DW | | |
| 3 | 3A | 3% 56–98 kDa PVA + 6% trehalose + 0.2% HRP + DW | 20/30 | by hand |
| | 3B | | 30/30 | |
| | 3C | | 30/35 | |
| | 3D | | 30/40 | |
| | 3E | | 30/50 | |

2.2.2. Electrospinning Process

Emulsion electrospinning was done using lab-scale electrospinning unit InoSpin from InoCure s.r.o. The humidity and temperature was controlled using the integrated temperature control unit InoCool from InoCure s.r.o. For all experiments, a cylinder (needle with the diameter G10) was used as an electrode. The fibers were collected using the rotary drum collector rotating at 500 rpm on a baking paper used to cover the aluminum surface. The distance between the needle and collector was 180 mm. Other settings used were flow rate (20 mL $h^{-1}$) and temperature and humidity inside the chamber (~22 °C and ~41%, respectively). The voltage used in each experiment is listed in Table 1.

2.2.3. Fiber Characterization—Scanning Electron Microscopy

Scanning electron microscopy (SEM) images were acquired by desktop scanning electron microscope from Phenom-World BV, using backscatter electron detectors (BSDs). Before analysis, samples were placed on pin holders with carbon tape and sputter coated using the SC7620 Mini Sputter Coater/Glow Discharge System from Quorum Technologies.

2.2.4. Protein Release Characterization

For burst release testing, samples from each electrospun scaffold (containing HRP) weighing 30–32 mg were placed in the 1.5 mL Eppendorf tubes and 1 mL of distilled water was added. The samples were placed in the refrigerator for 24 h. Then, the water solution was collected for the burst release characterization. The remaining fibers were dissolved in 0.5 mL of chloroform by mixing on Phoenix Instrument device RS-VF10 and protein was extracted with additional 0.8 mL of distilled water. After the separation of two phases, 0.4 mL of water was taken for characterization of protein in fibers. Less than 10% of the protein solution was lost in the process (measured by extraction from protein standard). Protein concentration characterization was performed using the Micro BCA™ assay kit according to the manufacturer's instructions. The quantitative data is presented as mean ± standard deviation (SD) of five independent replicates in each experiment.

2.2.5. Activity of the Encapsulated Proteins

Activity of the HRP was measured using the TMB substrate solution. After Micro BCA™ assay, samples collected after 24 h release were diluted to 5000 pg $mL^{-1}$; 100 μL of each sample where added to 100 μL of TMB substrate solution and mixed for 10 s. The absorbance of each sample was measured after 5, 10, and 15 min at $\lambda$ = 650 nm. Then, 100 μL of 2 M sulfuric acid was added to each well to stop the reaction and the absorbance was measured again at $\lambda$ = 450 nm. To determine protein activity in each sample, a standard curve with known active protein concentrations was used. The quantitative data is presented as mean ± standard deviation (SD) of five independent replicates in each experiment. Note, the activity of HRP was measured 48 h after scaffold production (experiments 1 and 2) or after 24 h (experiment 3). The activity loss of enzyme in the solution was examined experimentally and amounted to approximately 8% per 24 h (the measurement was done with three protein concentrations that include the minimum and maximum obtained during the release studies).

## 3. Results

Three factors that may influence protein activity during the emulsion electrospinning were investigated: (1) influence of various protein stabilizers, (2) influence of PVA degree of hydrolysis and molecular weight, and (3) influence of voltage. In all cases, fiber morphology, protein burst release, and activity were characterized. The summary of the samples were prepared and the experimental conditions involved are summarized in the Table 1.

### *3.1. Influence of the Protein Stabilizers in the Water Phase*

First, the influence of three selected stabilizers on the morphology of fibers, protein burst release from scaffolds, and protein activity was investigated. For this purpose, nonionic and hydrophilic surfactants or small molecules were used: PF68, PVP (polymer surfactants), and trehalose (sugar). The fibers were electrospun in conditions summarized in Table 1. The SEM images of the obtained fibers are shown in Figure 1(a). In all cases, it was possible to obtain a micro/nanofibrous mesh. However, more defects were observed when polymers surfactants were used, and, when trehalose was used, a scaffold without defects was made. The percentages of the proteins released from the fibers after 24 h in three cases are summarized in Figure 1(b). As shown, more than 60% burst release after 24 h was observed for samples with PF68 and PVP (samples 1A and 1B). Only the formulation with trehalose showed lower HRP release (45.5 ± 5%). However, as shown in Figure 1(c), very low activity (<15%) was measured in all cases. The lowest was for fibers containing PF68 (2.9 ± 0.5%) and the best result was obtained for the sample containing trehalose-12.6 ± 1.5%. Trehalose showed overall the best results and was used for all consecutive experiments.

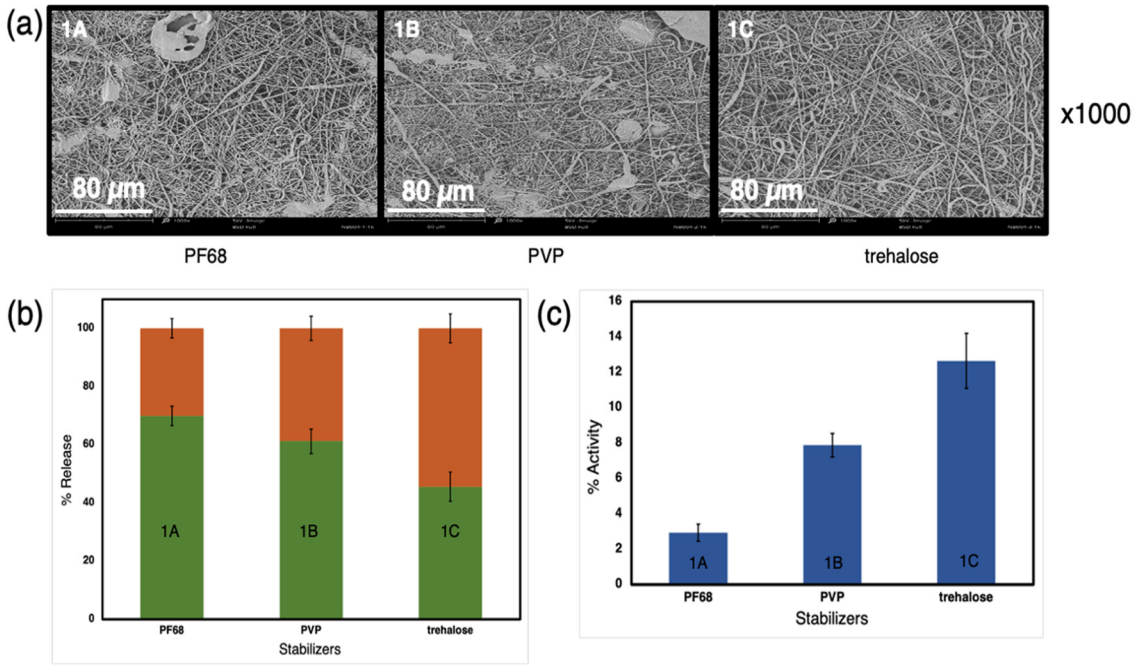


**Figure 1.** The influence of three stabilizers: 1A-PF68, 1B-PVP, 1C-trehalose on (**a**) the morphology of fibers; (**b**) HRP burst release from scaffolds; (**c**) HRP activity.

### *3.2. Influence of the PVA in the Water Phase*

The second experiment investigated the influence of different PVA molecular weights and the degree of hydrolysis on activity and burst release of proteins. Three PVA varieties with more than 98% hydrolysis, i.e., PVA 4–99 kDa, PVA 28–99 kDa, PVA 56–98 kDa, and three with 88% hydrolysis, i.e., PVA 5–88 kDa, PVA 26–88 kDa, PVA 40–88 kDa, were used. PVAs with a higher degree of hydrolysis are in general more hydrophilic and crystalline, while a low degree of hydrolysis indicate more hydrophobic and amorphous polymers. At the same time, the influence of three different molecular weights, low (4–99, 5–88), medium (26–88, 28–99), and high (40–88, 56–98) were also compared. In these experiments, the concentration of the PVA was kept the same, thus increasing molecular weight lead to the increase in the viscosity of the water phase and emulsion.

SEM images of obtained fibers are shown in Figure 2(a). Mixed (nanometer and micrometer) fibers were obtained in all cases. However, when higher molecular weight PVAs with lower degree of hydrolysis were used (PVA 26–88 and 40–88), there were large defects observed on the fibers. Emulsions with more hydrophilic PVAs led to a better quality micro/nanofiber scaffolds. Even with high molecular weight, PVA 56–98 kDa, and hence the high viscosity of the emulsion, the scaffold had a minimal number of defects. As shown in Figure 2(b), the PVA's degree of hydrolysis was also the major factor influencing the burst release of HRP. With more hydrophobic (samples 2D, 2E, 2F) less than 50% of the protein were released from fibers after 24 h. The lowest value was obtained for the sample containing PVA 26–88 kDA and it was 36 ± 1%. In samples where more hydrophilic varieties of PVA were present, the HRP release values were much higher and exceeded 70%. Finally, the activity of the released proteins was characterized and is summarized in Figure 2(c). In comparison to the sample 1C that contained the same amount of 5–88, only the samples with PVA 26–88 and 40–88 showed a higher activity of the encapsulated protein-13.2 ± 0.6% and 24.6 ± 0.4%, respectively. Interestingly, the samples with the highest activity, also showed the highest number of defects. For the further experiment formulation that did not produce, large defects were used, i.e., PVA 56–98.

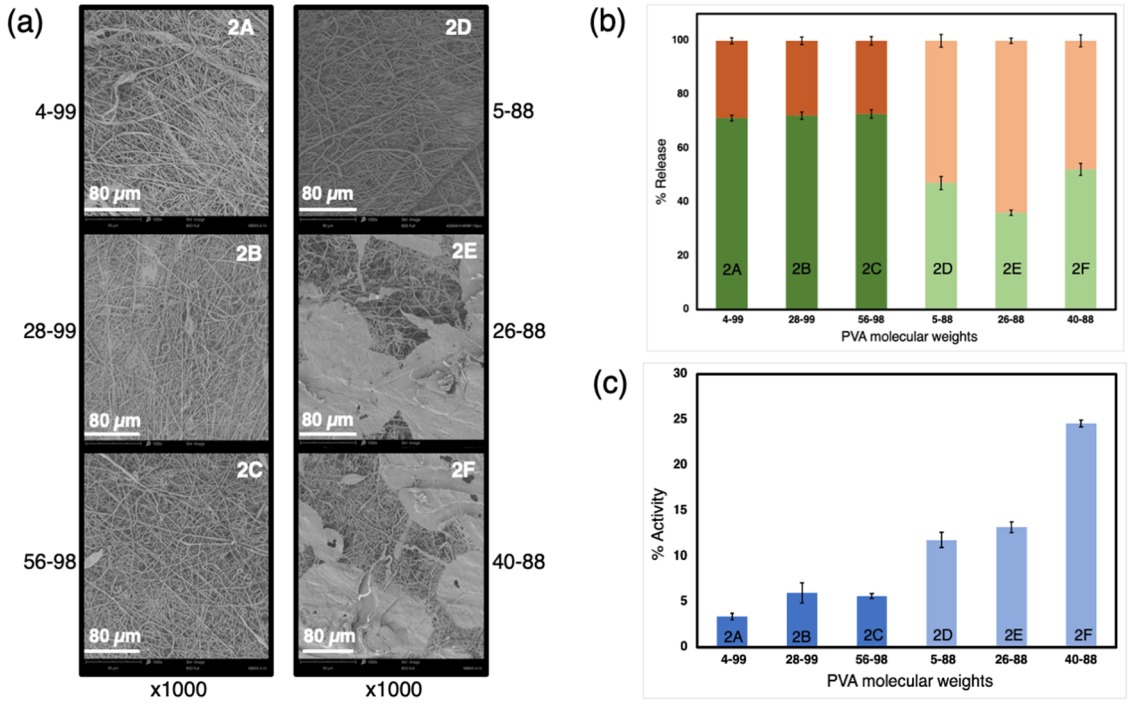


**Figure 2.** The influence of three more hydrophilic PVA varieties (2A-4–99, 2B-28–99, 2C-56–98) and three more hydrophobic PVA varieties (2D-5–88, 2E-26–88, 2F-40–88) on (**a**) the morphology of fibers; (**b**) HRP burst release from scaffolds; (**c**) HRP activity.

### *3.3. Influence of the Electrospinning Parameters on the Activity of the Proteins*

The third experiment analyzed the influence of electrospinning voltage on the activity of the protein. Moreover, to evaluate the potential loss of activity due to the shear homogenization, in this set of experiments, emulsions were prepared without the TURRAX homogenizer. The formulation was robust to the voltage changes from −20/+30 kV to −30/+50 kV, and, as shown in Figure 3(a), mixed nano-/micro fibrous scaffolds with only minor defects were obtained in all cases. The smoothest homogeneous fibers were obtained with −30/+40 kV that was used in previous experiments. As shown in Figure 3(b), electrospinning voltage does not seem to have a notable influence to the burst release. Comparable to the results discussed in the previous section, more then 70% of proteins were released after 24 h. There was also no correlation between the voltage used and the activity of the HRP (see Figure 3(c)). Importantly, compared to the samples in experiments 1 and 2, there was a significant three-fold improvement in the activity of all samples. The best result obtained for samples with medium voltages (30/30 and 30/35) were 40.6 ± 0.9% and 40.4 ± 0.8%, respectively. We attribute the significant loss of activity observed in previous samples to shear homogenization used in emulsion preparations.

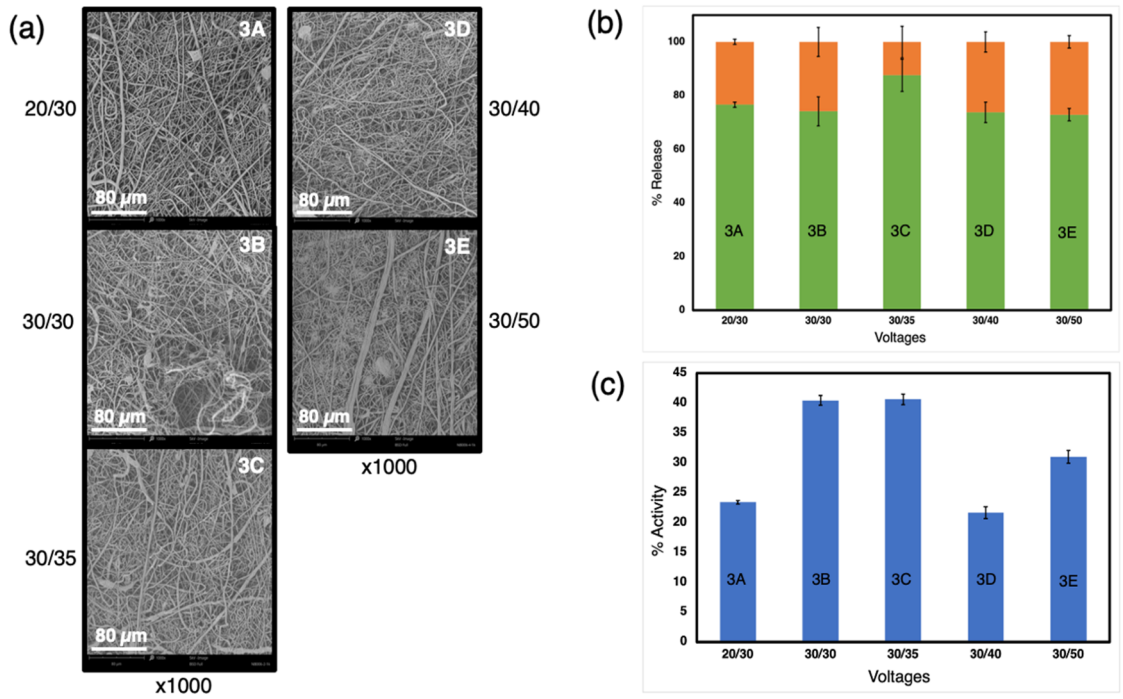


**Figure 3.** The influence of different voltages (3A-20/30, 3B-30/30, 3C-30/35, 3D-30/40, 3E-30/50) on (**a**) the morphology of fibers; (**b**) HRP burst release from scaffolds; (**c**) HRP activity.

## 4. Discussion

Electrospinning of scaffolds without defects is influenced by many different parameters, including the emulsion formulation and the settings of the electrospinning process [15]. The same parameters determine the subsequent properties of the proteins encapsulated in fibers, e.g., their bioactivity and release [16,17]. Our experiments demonstrated the importance of the formulation on both activity of encapsulated protein and its burst release. By changing the stabilizer used (see PF68 vs. trehalose), a four-fold increase in activity was observed. Further selection of the optimum excipient (PVA) allowed to increase the activity seven more times (from 3.4 ± 0.3% to 24.6 ± 0.4% for PVA 4–99 and 40–88, respectively). Finally, our results suggest that the most harmful step to proteins is the high shear homogenization that is often used to create the emulsion [12]. Preparing the emulsion by shaking, an increase in activity from 21% to 40% was observed. Multiple factors may lead to activity loss during the homogenization, e.g., protein exposure to high shear stress, exposure to the organic solvent, generation of smaller droplets. On the other hand, the burst release of proteins was mostly influenced by the use of PVAs with different degrees of hydrolysis. The burst could be reduced to below 40% when medium molecular weight PVA 26-88 was used. However, this formulation also leads to the large defects on the fiber mesh. To reduce the premature protein release from fibers, further research and optimization is needed. Better understanding of the influence of electrospinning parameters is also a subject of our further experiments.

## 5. Conclusions

We have investigated various emulsion formulations in order to increase the activity of the biomolecules (HRP) and decrease their burst release from the electrospun core-shell fibers. We have studied the influence of various stabilizers, PVAs, and electrospinning conditions (voltage used). Our results demonstrated the importance of appropriate stabilizers and emulsion preparation conditions on the activity of the encapsulated enzymes, and the role of the water phase excipients used on controlling the premature release of the protein. There was no obvious influence observed on neither activity nor release when the

electrospinning voltage was varied. However, this will require further experiments to verify. Better understanding of the factors influencing the emulsion electrospinning will certainly advance the applications of the method in drug delivery and tissue engineering.

**Author Contributions:** M.B. (Matej Buzgo) and A.S. conceived and designed the experiments; N.B. and M.B. (Magdalena Bartolewska) performed the experiments; N.B. analyzed the data; M.B. (Matej Buzgo) and A.S. contributed reagents/materials/analysis tools; N.B., M.B. (Magdalena Bartolewska), and A.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** This work was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreements No. 823981 (actiTox) and No. 824007 (iP-Osteo). N.B and M.Ba. have received support from the Erasmus+ traineeship programme for their research activities at InoCure s.r.o.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

API: Active Pharmaceutical Ingredients; PCL: Polycaprolactone; PVA: Poly(vinyl alcohol); PF68: Pluronic F-68; PVP: Polyvinylpyrrolidone; P31R1: Pluronic 31R1; HRP: Horseradish Peroxidase; HLB: Hydrophile-lipophile Balance; WP: Water Phase; OP: Oil Phase; DW: Distilled Water; BSD: Backscatter Electron Detector; SEM: Scanning Electron Microscopy.

## References

1. Yu, D.G.; Li, J.J.; Williams, G.R.; Zhao, M. Electrospun amorphous solid dispersions of poorly water-soluble drugs: A review. *J. Control. Release* **2018**, *292*, 91–110, doi:10.1016/j.jconrel.2018.08.016.
2. Yarin, A.L. Coaxial electrospinning and emulsion electrospinning of core-shell fibers. *Polym. Adv. Technol.* **2011**, *22*, 310–317, doi:10.1002/pat.1781.
3. Buzgo, M.; Mickova, A.; Rampichova, M.; Doupnik, M. Blend electrospinning, coaxial electrospinning, and emulsion electrospinning techniques. In *Core-Shell Nanostructures for Drug Delivery and Theranostics*, 1st ed.; Focarete, M., Tamprieri, A., Eds.; Woodhead Publishing: Cambridge, UK, 2018; pp. 325–347, ISBN 978-0-08-102198-9.
4. Sy, J.C.; Klemm, A.S.; Shastri, V.P. Emulsion as a Means of Controlling Electrospinning of Polymers. *Adv. Mater.* **2009**, *21*, 1814–1819, doi:10.1002/adma.200701630.
5. Vass, P.; Szabó, E.; Domokos, A.; Hirsch, E.; Galata, D.; Farkas, B.; Démuth, B.; Andersen, S.K.; Vigh, T.; Verreck, G.; et al. Scale-up of electrospinning technology: Applications in the pharmaceutical industry. *Wiley Interdiscip.* **2020**, *12*, doi:10.1002/wnan.1611.
6. Briggs, T.; Arinzeh, T.L. Examining the formulation of emulsion electrospinning for improving the release of bioactive proteins from electrospun fibers: Formulation of Emulsion Electrospinning. *J. Biomed. Mater. Res.* **2014**, *102*, 674–684, doi:10.1002/jbm.a.34730.
7. Qi, H.; Ye, Z.; Ren, H.; Chen, N.; Zeng, Q.; Wu, X.; Lu, T. Bioactivity assessment of PLLA/PCL/HAP electrospun nanofibrous scaffolds for bone tissue engineering. *Life Sci.* **2016**, *148*, 139–144, doi:10.1016/j.lfs.2016.02.040.
8. Place, L.W.; Sekyi, M.; Taussig, J.; Kipper, M.J. Two-Phase Electrospinning to Incorporate Polyelectrolyte Complexes and Growth Factors into Electrospun Chitosan Nanofibers. *Macromol. Biosci.* **2016**, *16*, 371–380, doi:10.1002/mabi.201500288.
9. La Mesa, C. Polymer–surfactant and protein–surfactant interactions. *J. Colloid Interface Sci.* **2005**, *286*, 148–157, doi:10.1016/j.jcis.2004.12.038.
10. Briggs, T.; Matos, J.; Collins, G.; Arinzeh, T.L. Evaluating protein incorporation and release in electrospun composite scaffolds for bone tissue engineering applications. *J. Biomed. Mater. Res.* **2015**, *103*, 3117–3127, doi:10.1002/jbm.a.35444.
11. Wang, Z.; Qian, Y.; Li, L.; Pan, L.; Njunge, L.W.; Dong, L.; Yang, L. Evaluation of emulsion electrospun polycaprolactone/hyaluronan/epidermal growth factor nanofibrous scaffolds for wound healing. *J. Biomater. Appl.* **2016**, *30*, 686–698, doi:10.1177/0885328215586907.

12. Tribst, A.A.L.; Cota, J.; Murakami, M.T.; Cristianini, M. Effects of High Pressure Homogenization on the Activity, Stability, Kinetics and Three-Dimensional Conformation of a Glucose Oxidase Produced by Aspergillus niger. *PLoS ONE* **2014**, *9*, doi:10.1371/journal.pone.0103410.
13. Schultz, S.; Wagner, G.; Urban, K.; Ulrich, J. High-Pressure Homogenization as a Process for Emulsion Formation. *Chem. Eng. Technol.* **2004**, *27*, 361–368, doi:10.1002/ceat.200406111.
14. Juttulapa, M.; Piriyaprasarth, S.; Takeuchi, H.; Sriamornsak, P. Effect of high-pressure homogenization on stability of emulsions containing zein and pectin. *Asian J. Pharm. Sci.* **2017**, *12*, 21–27, doi:10.1016/j.ajps.2016.09.004.
15. Yang, Y.; Li, X.; He, S.; Cheng, L.; Chen, F.; Zhou, S.; Weng, J. Biodegradable ultrafine fibers with core-sheath structures for protein delivery and its optimization. *Polym. Adv. Technol.* **2011**, *22*, 1842–1850, doi:10.1002/pat.1681.
16. Ji, W.; Sun, Y.; Yang, F.; van den Beucken, J.J.J.P.; Fan, M.; Chen, Z.; Jansen, J.A. Bioactive Electrospun Scaffolds Delivering Growth Factors and Genes for Tissue Engineering Applications. *Pharm. Res.* **2011**, *28*, 1259–1272, doi:10.1007/s11095-010-0320-6.
17. Shen, X.; Yu, D.; Zhu, L.; Branford-White, C.; White, K.; Chatterton, N.P. Electrospun diclofenac sodium loaded Eudragit® L 100-55 nanofibers for colon-targeted drug delivery. *Int. J. Pharm.* **2011**, *408*, 200–207, doi:10.1016/j.ijpharm.2011.01.058.