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Review

Recent advances in crystalline and amorphous particulate protein formulations for controlled delivery

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ABSTRACT

The number of particulate delivery systems for biologics is negligible compared to liquid dosage forms, signifying the complications associated with development of solid protein delivery systems. Particulate protein delivery systems can improve stability, reduce viscosity of suspensions at high protein concentration and allow for controlled drug release. This review discusses current advances in controlled delivery of particulate protein formulations. While the focus lies on protein crystals and delivery systems employing protein crystals, amorphous protein particles will also be addressed. Crystallization and precipitations methods and modifications allowing controlled delivery with and without encapsulation are summarized and discussed.

1. Introduction

With the increasing number of biologics in the pipelines of pharmaceutical manufacturers, approaches enabling improved stabilization and delivery of these molecules are increasingly sought-after. Administration of biologics via the gastrointestinal tract frequently yields poor bioavailability because of low intestinal absorption and enzymatic and chemical degradation of proteins prior to absorption, although some progress has been made in the case of peptides [1–3]. Consequently, parenteral administration is still the most common route of administration for protein pharmaceuticals. The vast majority of biologics for parenteral application nowadays are marketed as liquid or lyophilized formulations, but both presentations are associated with specific advantages and also some drawbacks and intricacies. Specifically, liquid presentations are convenient to use but require meticulous optimization of formulation composition, especially with regards to formulation pH, ionic strength and stabilizing excipients to achieve optimal physical and chemical stability [4]. Lyophilized biologic drug products apart from optimization of the formulation

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often require product-specific development or optimization of the lyophilization process to safeguard product quality while achieving commercially viable lyophilization process durations. However, lyophilization often results in high physical and chemical stability enabling shelf life of 3 to 5 years and may also be advantageous if highly concentrated solutions must be administered (up-concentration by lyophilization) [5,6].

Moreover, several additional requirements regarding tolerability and applicability of the formulation have to be factored in when developing liquid or lyophilized drug products. Beyond sterility, appropriate tonicity and pH-value, protein concentration is a main parameter, especially when dealing with high doses administered by subcutaneous or intramuscular injection, where injection volume is strictly limited. Apart from detrimental effects on physical stability, increasing protein concentrations frequently result in increased viscosity and/or opalescence affecting the injectability of the formulation and complicating visual inspection [7,8].

In addition to the challenges associated with development and stabilization of biologics formulations, standard liquid and lyophilized forms result in almost all cases in immediate drug release. Frequent application due to the short circulation half-life of numerous therapeutic proteins represents a significant burden for patients and sustained drug release would be beneficial. However, low drug load, protein degradation during encapsulation and low stability of the encapsulated protein during storage and after administration complicate development of polymeric protein delivery systems [9,10].

Crystalline protein formulations may represent an interesting alternative as protein crystals are densely packed allowing high drug loading, they have a reduced surface area reducing interactions with the solvent and polymeric scaffolds and show improved stability compared to amorphous formulations [11–13]. Furthermore, dissolution rate of protein crystals can be controlled without requiring encapsulation into a polymeric system [14]. Processing and administration of protein drug products also benefits from crystalline forms: viscosity of suspensions is substantially lower than that of equally concentrated protein solutions, allowing higher drug loading and simplifying administration [15]. In addition, interactions with aqueous or organic media are reduced and the protein stability at elevated temperatures is improved [16]. Despite these advantages, the crystallization of proteins with and without subsequent encapsulation for controlled delivery is still in its infancy, and previous reviews on this topic mainly have dealt with general suitability [17] as well as the upscaling and characterization of protein crystals [16].

In this review, we present the latest developments in the crystallization of pharmaceutically active proteins as well as give an update on the progress in delivery and encapsulation methods of amorphous protein precipitates and protein crystals.

2. Particle production methods

Defining optimal conditions for protein crystallization and precipitation can be tedious, and the transfer of crystallization conditions between molecules is typically unsuccessful, leading to the notion that protein crystallization is rather art than science. In order to prepare protein crystals, a protein solution has to be transferred into a thermodynamically unstable supersaturated state which returns to equilibrium by development of a crystalline or amorphous phase. For crystallization, it is the goal to increase the interactions between two protein molecules so that a well ordered arrangement takes place while nonspecific aggregation is avoided [18]. In general, the native conformation is maintained during and often preserved effectively after crystallization (see chapter 2.3.). There exists a wealth of knowledge about protein crystallization with focus on purification or structure determination [19] but much less research efforts were made toward manufacturing of larger batches. For elucidation of the protein structure, only a few large but almost perfect crystals are needed, typically produced in very small scale. However, in order to produce crystalline protein drug substance at commercial scale, batch crystallization methods appear to be the most suitable option (chapter 2.1.). The formation of protein particles is a wide field with countless methods published, a selection of which is presented in chapter 2.2.

2.1. *Preparation of protein crystals by batch crystallization*

Batch crystallization is the production of uniform crystals in a large scale, preferably with a high yield [20]. In general, the strategy is to quickly reach a high level of supersaturation of the protein so that numerous crystallization nuclei are formed simultaneously followed by a growth phase, whereby all nuclei grow in parallel, reaching the same size (Fig. 1A). This process is often initiated by a liquid–liquid phase separation between the protein and the solvent, followed by a first nucleation within the protein droplets [23]. According to the classical nucleation theory, more nuclei are formed if the difference of Gibbs free energy (ΔG) is largely negative, i.e. the system reaches a lower free enthalpy. With the free enthalpy ΔG being related to the chemical potential by $\mu = \left(\partial G / \partial n\right)_{p,T}$ systems tend to spontaneously 'escape' to lower chemical potentials as this leads to a reduction of the free enthalpy if other parameters, particularly the pressure p and the temperature T are held constant. Thereby, supersaturated solutions are thermodynamically instable as the chemical potential in the supersaturated state is higher as compared to the solid aggregate state – hence, these systems tend to aggregate with crystals frequently forming the lowest free enthalpy state. Accordingly, dissolved proteins in a supersaturated state spontaneously aggregate and thereby form nuclei on which further protein will deposit. The speed and extend at which supersaturation is reached strongly affects nuclei size and size distribution [24], but if the degree of supersaturation is pushed too high, precipitation takes place [20]. Commonly, supersaturation is reached by mixing a solution containing a high concentration of precipitating agent to a protein solution or by a rapid temperature drop. For the crystallization of individual proteins, different buffers and precipitating agents like salts, glycol, alcohols or poly(ethyleneglycol)s are required [25,26]. Decreasing the solvation of the protein is the primary goal for crystallization. For example, 'salting out' a protein by the addition of kosmotropes (e.g. sodium, lithium,

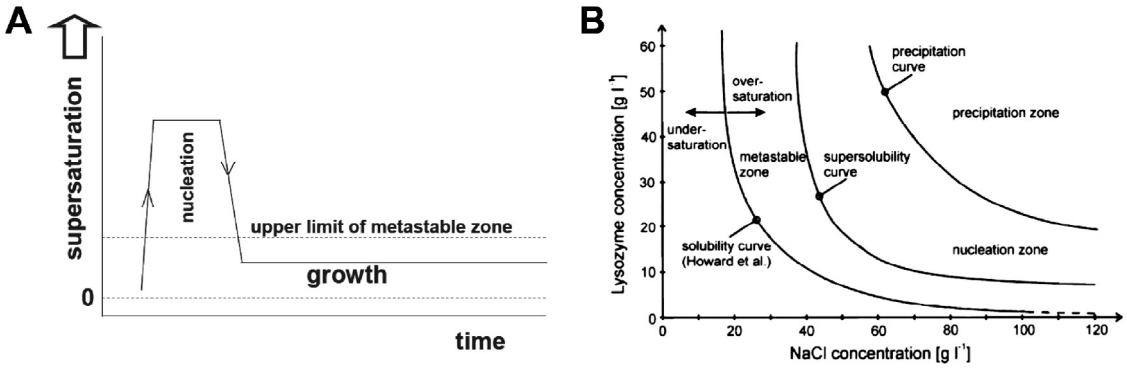

Fig. 1 – (A) Theoretical depiction of the time course of nucleation and subsequent crystal growth. Reprinted from [21] with permission from Elsevier. (B) Phase diagram of lysozyme crystallization. Reprinted and minimally adapted from [22] with permission from Elsevier. For efficient crystallization the aim is to surpass the supersolubility curve but to stay in the nucleation zone to avoid precipitation.

fluoride, sulfate or phosphate) has proven to be effective for crystallization [18].

The subsequent growth phase may take place within a few seconds up to several days [27]. As for all the other protein crystallization methods (e.g. sitting drop, hanging drop, etc.), individual screening and optimization is required for batch crystallization of proteins. For systematic screening, methods may be based on statistical design of experiments, allowing the efficient determination of main factors affecting crystallization. Protein concentration, type of precipitant, ionic strength, pH and temperature are important parameters to consider in such experiments. Moreover, Ostwald ripening may occur over longer periods of time, favoring formation of larger crystals if the solution is not being stabilized [28–30].

Batch crystallization conditions are frequently explored using water vapor diffusion experiments with subsequent transfer to micro-batch and to large batch crystallization [22,31]. A two or more dimensional phase diagram can be produced narrowing down the optimal conditions for crystal growth (Fig. 1B) and describing the interactions and influence of two or more variables on the solubility of the protein [32].

Insulin is probably one of the most prominent examples for protein crystallization with batch crystallization being still investigated and developed. One recent example is the study by Nanev et al. introducing a method for insulin batch crystallization [21]. Various crystal sizes between 18 and 57 μm with narrow size distributions could be achieved by high supersaturation of the protein solution. Insulin was dissolved in a citrate buffer at pH 7 in the presence of $ZnCl_2$ and acetone, and the solution was preheated to 50 °C. Nucleation was initiated by a rapid temperature drop. Parameters for the crystal size were insulin concentration, crystal growth time and growth temperature.

Crystallizing proteins by complexation with metal ions like Zn or other ionic molecules (e.g. protamine, ionic liquids) has not only proven to be effective but also to delay dissolution, offering the possibility to serve as a delivery system without further processing [14,33–36].

Recent advances in crystallization of pharmaceutically active drugs have been made in the field of monoclonal antibodies: trastuzumab, rituximab, infliximab [15] and anti-hTNF-alpha have successfully been crystallized [37,38]. Furthermore, batch crystallization of human growth hormone (hGH) was reported [39].

Apart from the potential advantages associated with the use of protein crystals as drug delivery system, batch crystallization of recombinant proteins and antibodies can be employed for NMR characterization or downstream processing. Such methods may develop into cost-effective alternatives to X-ray diffraction or Protein A or ion exchange chromatography, respectively in the future [40,41].

2.2. *Preparation of amorphous protein particles*

The production of amorphous protein particles can be achieved by mild processes that preserve the bioactivity of therapeutic proteins such as freeze drying, spray-drying, spray-freeze-drying and the precipitation in supercritical fluids. Freeze drying of a protein/PEG blend solution and subsequent removal of PEG from the matrix has proven to yield precipitated protein particles with homogenous size distribution [42]. Spray-drying of proteins is widely used for protein particle production, e.g. recombinant human anti-IgE and recombinant human deoxyribonuclease could be spray dried with and without addition of excipients and yielded in stable particles [43]. Spray-freeze drying is a modification of the standard spray drying process and offers the production of protein particles without the threat of degradation by heat. The sprayed protein droplets are collected in liquid nitrogen and are subsequently transferred to a freeze dryer [44,45]. Genentech and Alkermes developed a product consisting of hGH microparticles that were encapsulated in PLGA in amorphous form (Nutropin Depot). Particles of hGH were suspended in a solution of PLGA and spray-freeze-dried into liquid nitrogen.

Supercritical antisolvent precipitation technique (SAS) was demonstrated to provide protein particles by spraying solutions of various proteins dissolved in DMSO into concurrently flowing supercritical CO_2 [46]. Supercritical CO_2 acts as a solvent for DMSO but is a non-solvent for proteins (e.g. insulin, trypsin, and lysozyme) and thus leads to protein precipitation. After evaporation of the solvents, the protein particles can be collected. Advantages of SAS are the low processing temperatures as well as the low toxicity of excipients used in the process.

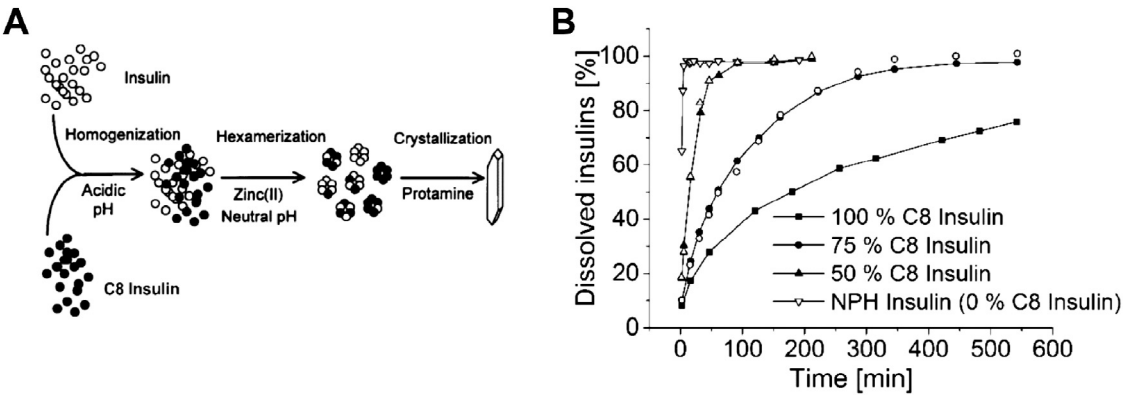

Fig. 2 – (A) Schematic drawing of co-crystallization of insulin with octanoyl (C8) modified insulin. (B) Dissolution rate of insulin crystals with different ratios of C8 insulin. Open symbols represent unmodified insulin, filled symbols C8 insulin. Reprinted by permission from Macmillan Publishers Ltd [53].

Further methods of protein precipitation are summarized in chapter 5.

3. Stability of protein crystals

Studies on the stability of protein crystals have been performed and discussed before [11]. For insulin, it was found that the dried amorphous form possess higher stability, whereas insulin crystals were found to be more stable suspended in aqueous media [47]. However, several other examples revealed an increased overall stability of crystalline proteins. Shenoy et al. compared the crystalline and amorphous forms (with and without stabilization agents like sucrose and trehalose) of glucose oxidase and lipase and found increased stability of the protein crystals [11]. Elkordy et al. obtained similar results for lysozyme [13].

The cause of the stabilizing effect of the crystalline state can be derived from the Hofmeister series. Effect of salts on stability of proteins in relation to the Hofmeister series has been investigated by Broering et al. [48]. With increasing concentration of kosmotropes, proteins are increasingly excluded from (aqueous) solvents, reducing potential risk of degradation. As mentioned above, kosmotropes are often found to promote protein crystallization, suggesting a relationship between the stabilizing effects of the kosmotropic ions and the desolvatization during crystallization. Collins evaluated the stabilizing effects of kosmotropes on proteins and underlined the relationship between the stabilizing effect and their capacity to crystallize proteins [18]. Protein stabilization and crystallization are both initiated by an exclusion of protein surface from the solvent forcing the protein to reduce its surface to a minimum. The structure having minimum surface usually represents the native state. This process can be continued until interactions between protein molecules lead to a well-ordered combination of more and more molecules to finally yield a crystal lattice in which all protein molecules are bound in their native state.

Frequently, organic solvents are used to encapsulate proteins into organic polymers. To prevent the crystals from dissolving during encapsulation, the solubility of proteins in the used solvents should be considered as the integrity protein crystals in the presence of organic solvents will only be maintained if dissolution is minimal. Chin et al. have shown for 34 solvent systems that solubility of lysozyme, a protein with high aqueous solubility, depends on hydrophobicity of the solvent (logP) and thus can be predicted for other solvents [49]. However, a certain aqueous layer is still required to maintain the native conformation of the protein, leading to the common recommendation that proteins should only be dried to a certain (maximally preserving) extent [50].

4. Delivery systems employing protein crystals

In principle, controlled release from protein crystals can be achieved either with or without encapsulation of crystals into a polymeric system. Since some protein crystals already show delayed dissolution encapsulation can be avoided in some cases. As an example, insulin crystals formed by complexation with zinc or protamine are capable of sustaining insulin release after subcutaneous application [51,52]. In contrast, encapsulation may protect proteins from destabilizing environmental conditions such as humidity, extreme pH and light.

4.1. *Unmodified protein crystals*

Brader et al. developed an insulin co-crystal composed of human insulin and octanoyl modified insulin [53]. The modification with octanoyl residues was achieved by covalent conjugation to LysB29 of human insulin. Thereby, hydrophobicity was increased without affecting bioactivity. Co-crystallization of unmodified and modified insulin at different ratios yielded insulin crystals with overall prolonged dissolution behavior (Fig. 2).

Similarly, recombinant human interferon α-2b (rhIFN) chelated with Zn ions showed sustained release from protein crystals [54]. Crystals were produced by hanging drop vapor diffusion method in the presence of zinc acetate and sodium acetate. Several morphologies were obtained by varying pH, ionic strength and the addition of further precipitants like PEG of

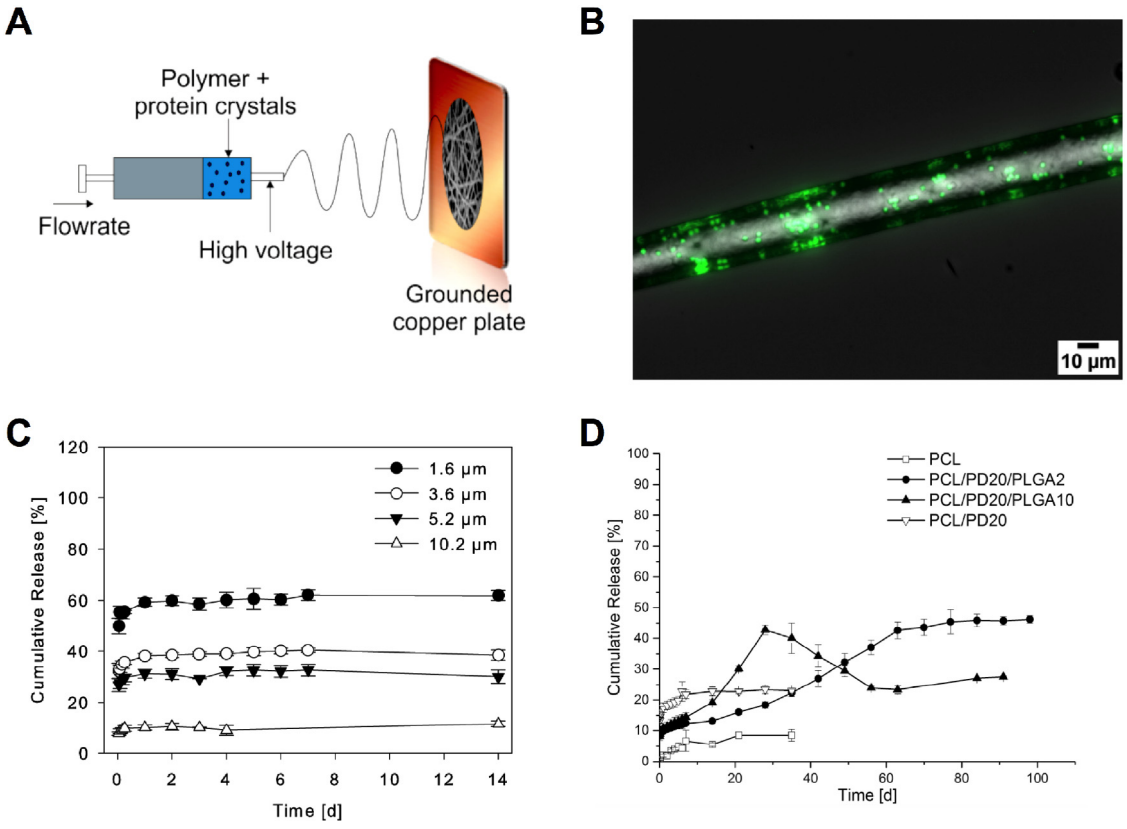

Fig. 3 – (A) Schematic depiction of encapsulation of protein crystals by electrospinning. (B) Micrograph of distribution of FITC labeled lysozyme crystals incorporated within a PCL fiber with a loading of 5% m/m. (C) Cumulative lysozyme release from electrospun nonwovens with different fiber diameters while keeping lysozyme crystal size constant (2.1 µm). (D) Cumulative lysozyme release from electrospun nonwovens with different polymer compositions. (C) Reprinted with permission from [55]. Copyright (2014) American Chemical Society. (D) Reprinted from [56] with permission from Elsevier.

different molecular weight. Biological activity of rhIFN was retained and *in vitro* dissolution showed an initial burst over 8 h, followed by a subsequent release over up to 48 h.

Shi et al. developed a method for batch crystallization of rhIFN [35]. In this study, a semi-crystalline structure was formed by molecular self-assembly of the protein and protamine sulfate in the presence of zinc acetate. Spherical, monodisperse semi-crystals were obtained with adjustable ratios of protamine in the semi-crystal. Dissolution depended on the relative amount of protamine in the particles. Increasing ratios of protamine to rhIFN resulted in reduced dissolution of crystals, achieving release for up to one week. In vivo experiments confirmed the delayed dissolution, resulting in extended blood levels for up to seven days for the highest protamine to rhIFN ratio.

4.2. *Protein crystal encapsulation*

Human growth hormone (hGH) crystals were obtained by batch crystallization using two different approaches [39]. Admixing sodium acetate and PEG 6000 and incubating over 12–16 h at 33 °C produced circular crystals with yields over 90%. In contrast, crystallization of hGH with zinc and acetone as precipitating agents for 21–24 h at 15 °C resulted in hexagonal crystals (yield > 50%). Crystals were coated either with the poly(arginine) or protamine simply by overnight incubation in the crystallization medium. Protein structure and activity could be completely retained. After coating of crystals, *in vitro* dissolution was significantly prolonged. However, no differences between the two coatings were observed. *In vivo* tests of poly(arginine) coated hGH crystals in monkeys revealed elevated serum levels of hGH for about 7 d and an increased induction of IGF-1 serum levels compared to soluble hGH.

Crystallized lysozyme was encapsulated by Puhl et al. into nonwovens by dispersing the crystals in organic poly(caprolactone) (PCL) solution followed by electrospinning of the suspension (Fig. 3A) [55]. Electrospinning results in an immensely increased and well controllable surface of the delivery systems and allows generation of various macroscopic morphologies optimally adapted to the site of application. Protein crystals withstood the encapsulation process and were found to be located discretely within the electrospun fibers (Fig. 3B), and loading could be adjusted between 0.25 and 5% (m/m). It was shown that release depended on the size of the protein crystals and the diameter of nonwoven fibers as well as the polymer composition. Burst release was controlled within broad margins by varying the crystals size and the fiber diameter (Fig. 3C). It was found that higher ratios of fiber diameter to crystal size resulted in reduced burst release. Moreover, the addition of polidocanol to the polymer matrix prior to

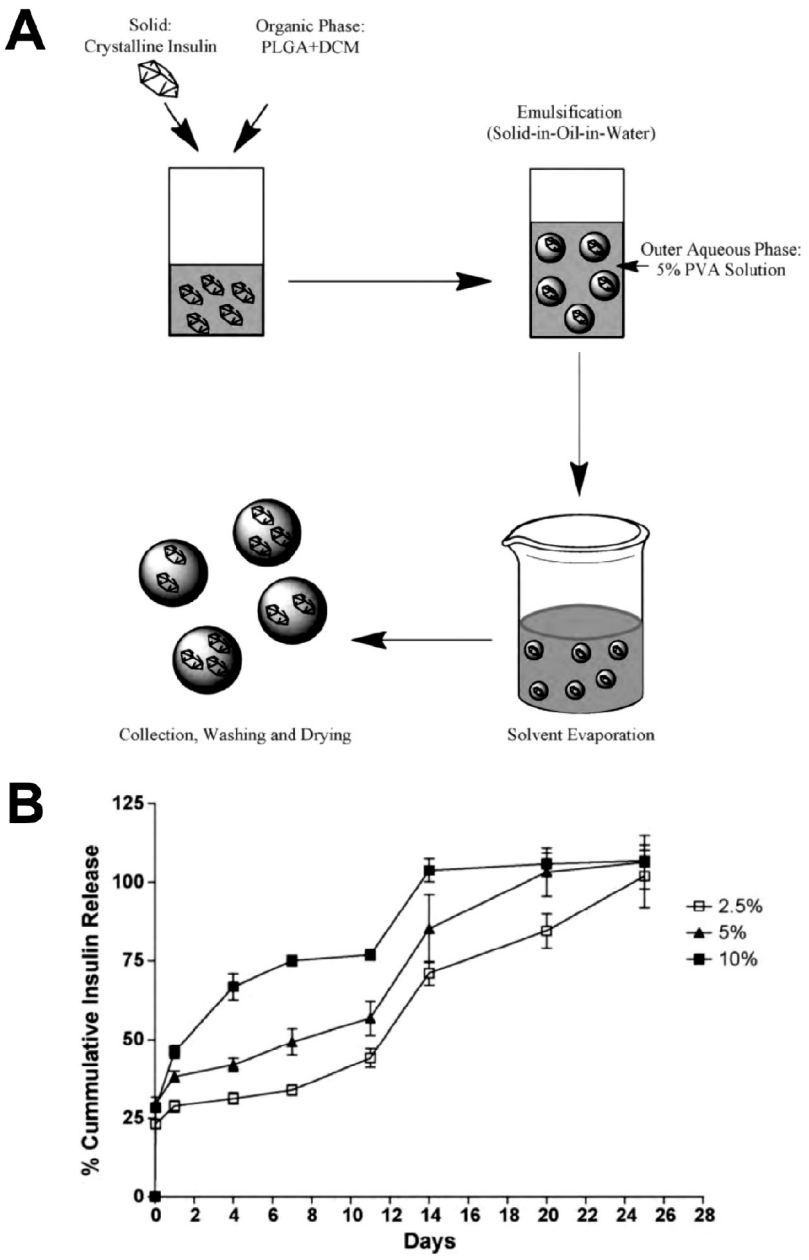

Fig. 4 – (A) Encapsulation of insulin crystals into PLGA microcapsules by solvent evaporation from an S/O/W system. (B) Cumulative insulin release from PLGA microspheres with different loading % m/m. Reprinted from [57], with permission from Elsevier.

electrospinning increased overall release, altering the release mechanism. Release rate modification was achieved by the addition of PLGA for controlled release over several days (Fig. 3D) [56].

Crystalline recombinant human insulin was encapsulated in PLGA microspheres by a solvent extraction method by suspending insulin crystals in PLGA dissolved in DCM [57]. By dispersing this system in an aqueous solution with 5% PVA, a solid-in-oil-in-water (S/O/W) suspension was formed. After DCM evaporation, insulin loaded PLGA microspheres could be collected (Fig. 4A). Loading was adjustable between 2.5 and 10%, and encapsulation efficiency ranged between 99 ± 10% and 78 ± 1%, at 2.5 and 10% loading, respectively. The release rate increased with higher loading and exhaustive release was reached after 2–3 weeks (Fig. 4B).

5. Delivery systems employing non-crystalline protein particles

Particulate protein delivery is a very broad field, and frequently it is not possible to identify the exact physical state of protein particles (i.e. amorphous or crystalline). However, protein particles share some of the advantages of protein crystals (e.g. dense packaging and reduced surface area). In addition,

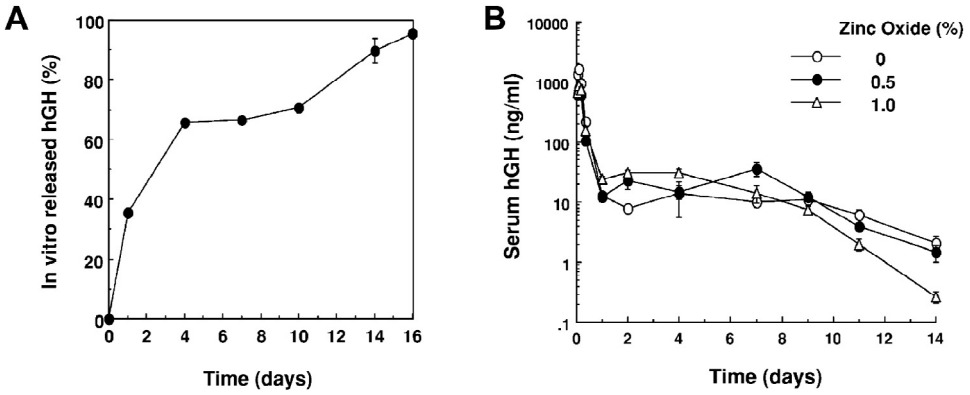

Fig. 5 – (A) Cumulative hGH release from PLGA microspheres with 7% protein loading. hGH was precipitated with 0.5% m/m zinc oxide. (B) Serum hGH levels in immunosuppressed rats after a single s.c. injection of hGH loaded PLGA microspheres with different amounts of zinc oxide. Reprinted from [63], with permission from Elsevier.

encapsulation technologies suitable for protein particles may be transferrable to crystalline proteins. Differences between simple protein particles and protein crystals are likely to be found in dissolution rate and thermodynamic stability.

Significant research and development efforts in this field were focused on insulin delivery through inhalation. Exubera®, a spray dried insulin formulation for pulmonary application, was the first marketed product but has been taken off the market due to insufficient acceptance and disappointing sales figures [54]. MannKind Corporation is the only company further pursuing inhaled insulin delivery at the moment. In June 2014, MannKind was able to get its fast acting inhalable insulin (Afrezza®) approved by the FDA [58]. Major improvements of inhaler technology resulting in significantly smaller inhaler design than Exubera® in conjunction with its high efficacy and rapid onset of action is hoped to result in a commercially viable product [59].

However, the production and application of spray-dried protein particles is a topic of its own and does not necessarily comprise the purpose of protein delivery as a particulate system. Detailed information on spray drying of proteins the reader is referred to [60]. Herein, we discuss spray-dried protein particles only in cases where obtained particles were intended for drug delivery to the human body and not only prepared for stabilizing reasons.

5.1. *Unmodified non-crystalline protein particles*

Under normal conditions, amorphous protein particles dissolve rapidly and therefore do not allow sustained release. Consequently, protein particle production without further processing is mainly applied when pulmonary application is intended.

As a typical example, recombinant human insulin particles suitable for pulmonary application were produced by precipitation of insulin and subsequent spray-drying of the resulting suspensions [61]. First, protein particles of different sizes were produced by the addition of various amounts of methanol to an aqueous insulin solution under stirring. Subsequently, either suspensions of these particles or a particle free protein solution were spray-dried to yield a free-flowing powder with particle sizes of approx. 1–5 μm. The largest precipitates kept their original size after spray-drying while smaller particles agglomerated during spray-drying. The aerodynamic behavior was investigated with a New Generation Impactor (NGI), and all preparations were found to be superior compared to Exubera®. While all insulin particles from the spray-dried suspensions had no significant difference in aerodynamic behavior, the spray-dried solution had the smallest fine particle fraction, which is considered to reach the lung.

5.2. *Encapsulation of non-crystalline protein particles*

Human growth hormone (hGH) particles were produced by lyophilization of buffered protein solution with addition of zinc acetate [62]. Zinc was used to reduce the solubility of hGH. The lyophilisate was densified, followed by regrounding to a powder. After sieving through a 212 μm screen, protein particles with defined size were obtained. Both the precipitation with Zn and the densification reduced the dissolution rate significantly. Untreated hGH particles dissolved instantaneously, while complete dissolution of the densified particles required 20 min and complexation with Zn prolonged the dissolution time-scale to hours. For encapsulation, PLGA was dissolved in either 1-methyl-2-pyrrolidone, triacetin, ethyl benzoate or benzyl benzoate and the protein particles were dispersed therein. Injection of these suspensions in aqueous medium produced PLGA gels, which served as a protein particle depot. Release was evaluated *in vivo*, and serum hGH levels were measured. After an initial burst the release lasted up to 28 d.

In another study, lyophilized recombinant human growth hormone powder was encapsulated in PLGA by S/O/W method [63]. PLGA was dissolved in dichloromethane and the protein powder was dispersed therein. Injection in water/poly(vinylalcohol) generated an S/O/W emulsion from which DCM was evaporated under stirring until particles were obtained. Particles larger than 74 μm were removed and a powder suitable for s.c. injection was obtained. Complete protein release *in vitro* was achieved within several weeks (Fig. 5A). *In vivo* release behavior of different formulations with ammonium acetate and ZnO as well as different PLGA types was investigated, and elevated serum hGH concentrations were observed for all formulations over approximately 16 d (Fig. 5B).

Hen egg lysozyme particles were incorporated in an in situ forming biodegradable microparticle system (ISM) [64]. The protein was dissolved together with PLGA either in pure dimethylsulfoxide (DMSO) or DMSO with ethyl acetate and water. Ethyl acetate precipitated lysozyme and thus in dependence of the solvent composition either dissolved or homogeneously precipitated lysozyme was incorporated. These solutions/suspensions were emulsified into sesame oil. After injection into aqueous medium, a homogeneous microparticle system was produced. The protein was released over approximately two weeks, and bioactivity was almost completely retained while the precipitated lysozyme showed slightly greater activity compared to the unprecipitated protein.

6. Conclusions

Administering proteins in the crystalline or particulate state represents a versatile and suitable mode of protein delivery. However, finding the right crystallization conditions usually is expensive and time consuming and probably not every protein can be crystallized. This investment may pay off though, considering the benefits of storage and processing stability. In most cases, the crystalline state surpasses the amorphous particulate state in terms of thermodynamic and physical stability. Moreover, crosslinking or crystallization with excipients like protamine may further increase stability and reduced energy within the crystal lattice may reduce dissolution rates offering delivery without further encapsulation.

If crystalline protein cannot be obtained, amorphous particulate protein represents an alternative. Thereby, high loading and reduced surface area in contact with potentially harmful environments can also be achieved.

Producing particulate protein for delivery, both crystalline and amorphous, is still a rather unnoticed field. With growing expertise on protein crystallization methods as well as protein stability itself, delivery is coming more and more into the focus of the formulation scientists and protein crystallization is slowly moving away from being solely used for purification and structure analysis. While momentarily the limits caused by the costs and efforts to be invested often prevail the benefits, this may change with increasing knowledge in protein batch crystallization and thus decreasing hurdles for the exploration of new crystallization protocols.

As soon as uniform crystals or precipitated powder is obtained, well established encapsulation methods can be applied. S/O/W solvent deposition, electrostatically driven coating and electrospinning have proven to be applicable.

Release rates are well controllable although an exhaustive release is not always given for larger macromolecules because their diffusion in polymer matrices is slow. Bioactivity and *in vivo* experiments underline the compatibility of crystalline and particulate protein formulations for controlled delivery.

Finally, another major point has to be considered: the rationale why one would seek a long term stabilized protein formulation with complex release mechanisms. A conventionally designed protein formulation is very well capable to maintain the protein's integrity over a sufficient timespan. Either protein solutions with the addition of stabilizing agents or lyophilized protein powders that are reconstituted in premixed buffers prior to application, mostly offer effective stabilization. Besides that *in vitro* stability and *in vivo* half-life differ from protein to protein. The nature of monoclonal antibodies mostly grants them a long circulation in the human body even without controlled release mechanisms. Peptide hormones on the other hand often need frequent administration, sometimes even several times a day. For tissue engineering, growth hormones benefit from a site directed and controlled release. Consequently, for the former group, the development of a drug delivery system is far less useful, while for the latter group, efficient drug delivery would significantly improve patients' lives.

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