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Research paper

Formulation of an intermediate product from human serum albumin for the production of a solid dosage form

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Abstract

The main objective of this study was to evaluate and to increase the processibility of a model protein (human serum albumin (HSA)) for preparation of an intermediate for a solid dosage form. The applicability of the solid forms is easier, and therefore their formulation is a promising method for the application of proteins. The layering of powdered cellulose with HSA solutions of different concentrations in a fluid bed apparatus with the top spray method was applied. The yield of this technique was very good, independently of the concentration of the applied solution. The HSA covered the particles (the HSA layer formed was smooth), but it caused aggregation of the cellulose particles, and spray-dried microparticles also formed. The proportion of optimum-sized particles (200–315 μm) decreased. The largest amount was detected for the samples prepared with liquid containing 15% HSA (about 2 times higher than the second best). Not only the size, but also the shape of the particles was changed. The alteration in this parameter caused a change in the flowability. This was likewise the best for the samples prepared with the liquid containing 15% HSA. The concentration of HSA in the fraction containing smaller particles was higher, because of the abrasion of the particles and the enrichment of the spray-dried HSA. The distribution of HSA in the large particles was uneven. The layering of powder cellulose can be applied to produce an intermediate from HSA for solid dosage forms, but the appropriate concentration of this protein solution must be optimized previously because HSA can act as a binder. The formation of large agglomerates must be eliminated, because the distribution of the active agent in these is very inhomogeneous. The present results indicated that the best value can be achieved with liquid containing between 12.5% (most homogeneous distribution of HSA) and 15% HSA (best flowability).

Keywords: Human serum albumin; Fluidized bed technology; Layering; Powdered cellulose; Solid dosage form

1. Introduction

Biologically active peptides and proteins are increasingly becoming a very important class of therapeutic agents because of their extremely specific activity and high tolerability by the human organism [1]. Their rapid clearance in the body necessitates repeated injections, which is an inconvenient form of therapy, as well as being painful [2]. It is therefore reasonable to formulate dosage forms which can be applied by the patient without pain. Alternative routes for the systemic effect are currently becoming widespread. Thus, transdermal, rectal, nasal and buccal therapeutic systems can be used without the destructive effects of the gastrointestinal tract on the proteins [3–5]. The dosage forms can be liquid, semisolid or solid (tablets or capsules) with an appropriate bioadhesive effect [6–8]. The applicability of these solids (e.g., oral, tablets for buccal or sublingual use) is easier, and therefore their formulation is a promising method, though with many challenges.

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One possibility for tablet-making is direct compression [9,10]. The preparation of appropriate materials from the component present in a liquid (e.g., serum) for this method is very difficult because there are many important parameters (e.g., good flowability and compressibility) which must be considered [11]. Another problem with direct compression is the higher compressing force, and so higher temperature during the compression can be reached which can destroy the proteins. The larger particles of protein can arrange into "hot spots", where the temperature is higher [12–14]. However, the processibility of small particles is very difficult because of the autoadhesion [15,16]. The preparation of appropriate intermediates can promote the preparation.

The intermediate products can be different granules or particles prepared by the layering technique. This layering method is nowadays becoming more popular for the preparation of multiparticulate dosage forms, because conventional granulators (fluidized bed, centrifugal, etc.) can be applied and the small amount of active ingredient can be processed within a short time [17–19]. This technique conventionally uses an inert spherical carrier (e.g., sucrose or cellulose) with the same diameter. The compressibility of these spheres (non-pareils) is not appropriate, and it cannot be relevantly changed with other additives. Accordingly, other carriers must be used for the tablet-making. Powdered cellulose can be a promising component because it is inert and compressible with other cellulose derivatives, but the surface of this component is uneven.

The main objective of our study was to evaluate and to increase the processibility of model protein (human serum albumin (HSA)) in order to prepare an intermediate for an oral solid dosage form. HSA is a single-chain protein synthesized and secreted from the liver cells. HSA (M_w 66,472 Da) is found mainly in plasma (~50%) where it maintains the pH and osmotic pressure and has a major role in transporting a wide range of molecules such as metals, fatty acids, amino acids, metabolites and many drugs (e.g., interferon) [20–22]. It is known that HSA is sensitive to heat, high shear mixing and ions, etc. [23–25]. The choice of appropriate formulation (additives and methods) is therefore very difficult. A method was used which is appropriate for scaling-up. One of the available options was the HSA coating of a carrier in a fluid bed apparatus with the top spray method. Since there is no incompatibility between cellulose and HSA, powdered cellulose was used. This material is often utilized as a functional filler for tablet-making [26]. Many aspects must be considered during fluidization. In this part of our work, the concentration of the liquid was chosen as an evaluated factor. The information on this parameter is necessary to optimize the process of converting HSA into a solid dosage form and to determine the critical control points. The aim was to prepare a suitably flowing, even-covered intermediate with minimum agglomeration formation. The flowability and particle size of the products were therefore evaluated, and they were classified into three groups according to their size. The contents of active agent in the different groups were also tested.

2. Materials and methods

2.1. Materials

A solution containing 39.9 g/l of HSA (and also NaCl, KCl, Na_2HPO_4, KH_2PO_4, etc.) was used in this work (Trigon Biotechnological Ltd, Budapest, Hungary). Parameters of liquid were as follows:

Content of albumin: min. 98% of the total protein
Content of endotoxin: 5 IE/mg
Content of chloride: 4.85–5.35 mg/ml
Osmolarity: 285.0–315.0 mOsmol/kg

This liquid was lyophilized at −50 °C and 180–200 mTorr with a freeze-drying machine (Flexi-Dry MP FTS Systems, Inc., Stone Ridge, USA). The lyophilized products were dissolved in the original liquid (39.9 g/l) to produce liquids with contents of 5%, 10%, 12.5% 15% and 20%. The carrier was powdered cellulose with an excellent flowability and superior stability (Arbocel A 300, JRS GmbH&Co, Rosenberg, Germany).

2.2. Preparation of samples

The samples were prepared with a fluid bed apparatus (Strea-1, Niro-Aeromatic AG., Bubendorf, Switzerland). The top spray method was used. The concentration of the layering liquid was varied (see above). The quantity of HSA applied was the same, and several other factors were also varied for the sample. First, 100 g of powdered cellulose was treated with 300 g of water to evaluate the effect of water on the aggregation. It is known that the average denaturation temperature of HSA is about 60 °C so the drying temperature was lower [25]. The constant parameters were as follows:

Quantity of carrier: 100 g
Nozzle diameter: 0.8 mm
Drying temperature: 35 °C
Flow rate: 4 ml/min
Atomizing pressure: 2 bar
Blow-out pressure: 4.5 bar
Air volume: 40 m^3/h
RH of the input air: 45%
Preheating: 8 min without atomizing air
Process: in blocks (10-min atomization + 2-min drying)
Last drying: 8 min without atomizing air
The varied factors are listed in Table 1.

2.3. Morphological study

A Hitachi S2400 (Hitachi Scientific Instruments Ltd, Tokyo, Japan) scanning electron microscope was used to determine the shape and the surface of the particles. A sputter coating apparatus, Polaron E5100 (Polaron Equipment

Table 1
Parameters in preparation of samples

Sample	Concentration of HSA solution (%)	Quantity of the liquid (g)	Total process time (min)
S0	0	300	134
S1	5	300	130
S2	10	150	65
S3	12.5	120	50
S4	15	100	47
S5	20	75	33

Ltd, Hertfordshire, England), was applied to induce electric conductivity on the surface of the sample. The air pressure was 1.3–13 mPa.

2.4. Particle size distribution

The sizes and the size distributions of the samples were evaluated. An analytical sieve (Retsch GmbH, Haan, Germany) was used. D50 was determined with sieving system software (Retsch EasySieve 2.0).

Depending on the particle size, the products were divided into three groups (<200, 200–315 and >315 μm).

2.5. Flow properties

A powder testing apparatus (PTG-1, Pharma Test GmbH, Hainburg, Germany) was used to test the flow time of 100 ml of samples. A teflon accessory 10 mm in diameter and stirring at 25 rpm was applied. Three parallel experiments were performed.

2.6. Determination of HSA content

The concentration of HSA was determined with a UV spectrophotometer (Unicam Heλios Alpha, Spectronic Unicam, UK) at 562 nm. The Micro BCA™ Protein Assay (Pierce, Rockford IL, USA) was applied for the determination. The Micro BCA Protein Assay combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by bicinchoninic acid. The first step is the chelation of copper with protein in an alkaline environment to form a blue colored complex. In this reaction, known as the biuret reaction, peptides containing three or more amino acid residues form a colored chelate complex with cupric ions in an alkaline environment containing sodium potassium tartrate. In the second step of the color development reaction, BCA, a highly sensitive and selective colorimetric detection reagents reacts with the cuprous cation (Cu^{1+}) that was formed in step 1. The purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion. The BCA/copper complex is water-soluble and exhibits a strong linear absorbance at 562 nm with increasing protein concentrations [27].

The test fluid was phosphate buffer, pH 7.2. The concentrations of active agent in the different groups were determined. The calculated value was 13.04% (15 g/(15 g + 100 g)).

3. Results

3.1. Effect on yield

After the preparation of the intermediates, the yields were calculated. It can be seen that the yields of the samples containing HSA were very good (Table 2). The value for S0 was the lowest. The long treatment of this sample could cause a higher ratio of lost particles through the filter system. This was not detected for the other samples, which can be explained by the increase in their particle size, so that the possibility of loss was lower.

3.2. Effect on shape

It can be seen from the SEM pictures that the surface of the original Arbocel A 300 was irregular (Fig. 1). The sticking of the treated particles is clearly visible, with the irregular surface of the cellulose with binder layers of HSA forming bridges between the particles (Fig. 2). A covering layer (''film-like'') of HSA was detected at higher magnification. Numerous HSA spheres 1–2 μm in diameter were also produced during the process (Figs. 3 and 4). This

Table 2
Yield of preparation

Sample	Yield (%)
S0	73.04
S1	96.35
S2	94.26
S3	97.3
S4	94.52
S5	95.39

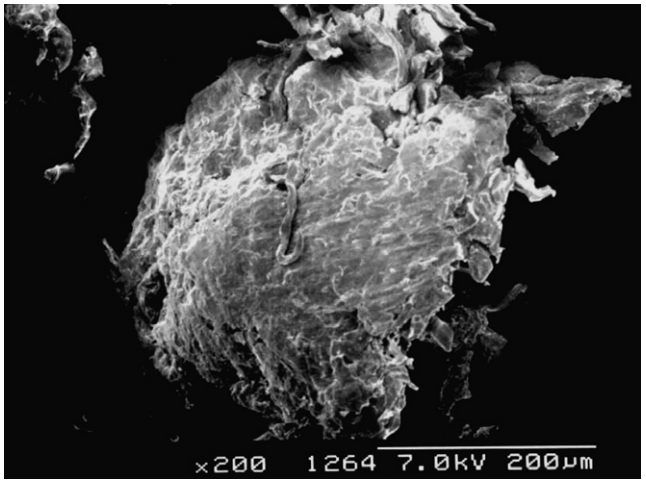

Fig. 1. Arbocel A 300 (SEM 200×).

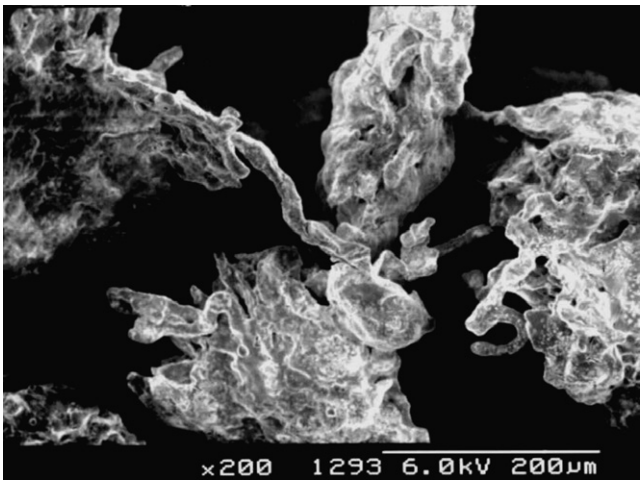

Fig. 2. S4 particle <315 μm (SEM 200×).

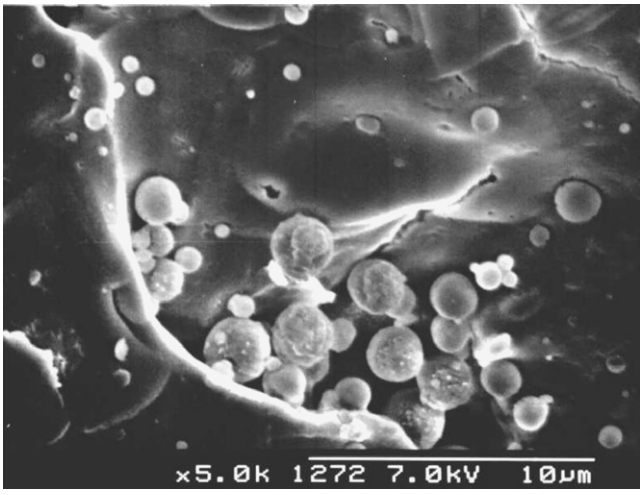

Fig. 3. Surface of S4 (SEM 5000×).

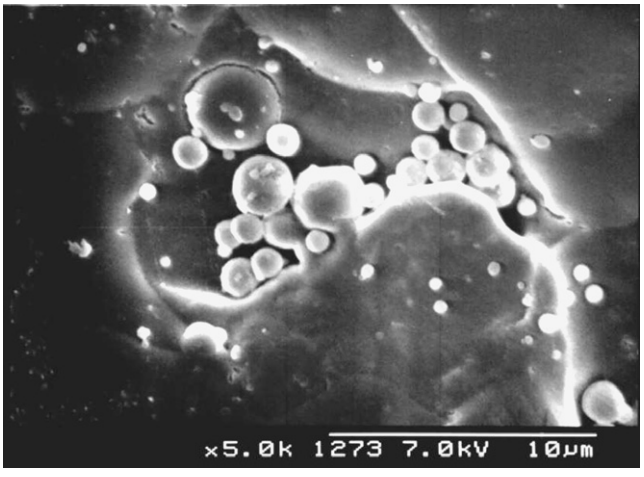

Fig. 4. Surface of S4 (SEM 5000×).

was a consequence of the spray-drying of the solution. These microparticles settled mainly in the irregularities in the powdered cellulose. Because of the size of these particles (smaller than the filter), the loss during the process can be relevant. The covering of the cellulose was very smooth, but a few defects could be detected. These can be due to the incorporation of the spray-dried particles into the layer, and the rupture of the HSA layer and the grained surface (because of the recrystallization of salts) (Figs. 5 and 6).

3.3. Effect on particle size

Because of the previous results, the particle sizes were also determined. It can be seen that the application of HSA caused an increase in the size of the particles (Table 3 and Fig. 7). These results emphasized that HSA was a binder, since the coating could not cause an increase of more than 50% in the diameter. There was no obvious relationship between the concentration of the liquid and the particle size. This can be a result of the complexity of the process. It is known that the viscosity of HSA solution is changing with the concentration [28]. At the lowest concen-

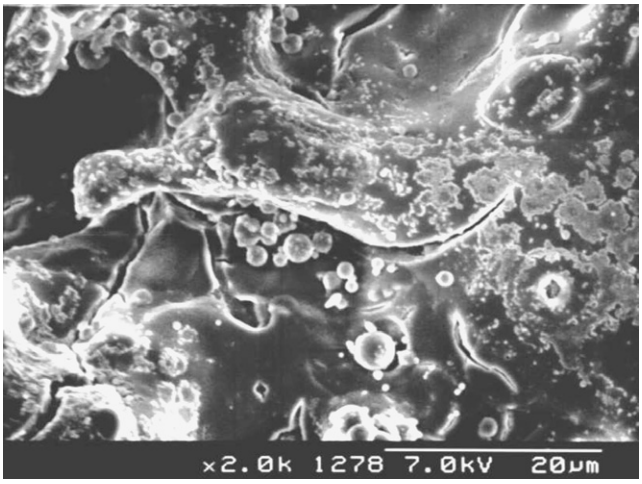

Fig. 5. Surface of S4 (SEM 2000×).

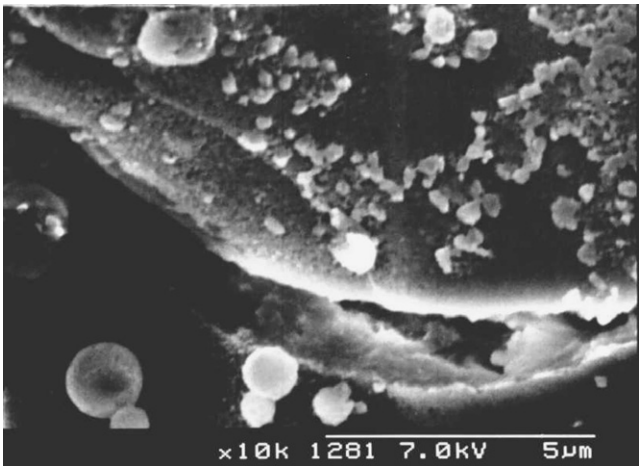

Fig. 6. Surface of S4 (SEM 10000×).

Table 3
Properties of samples

Sample	D50 (μm)	<200 μm	200–315 μm	>315 μm	Flow time (s)
Arbocel A 300	277	10.3	67.9	21.8	13.4 ± 1.6
S0	258	21.3	67.0	12.7	7.7 ± 0.2
S1	466	5.0	10.8	84.3	105.5 ± 26.0
S2	452	4.1	13.6	82.5	46.8 ± 7.9
S3	467	1.9	10.7	87.4	73.4 ± 10.9
S4	406	3.7	23.4	72.9	26.5 ± 5.5
S5	465	1.7	12.4	85.9	231.3 ± 29.1

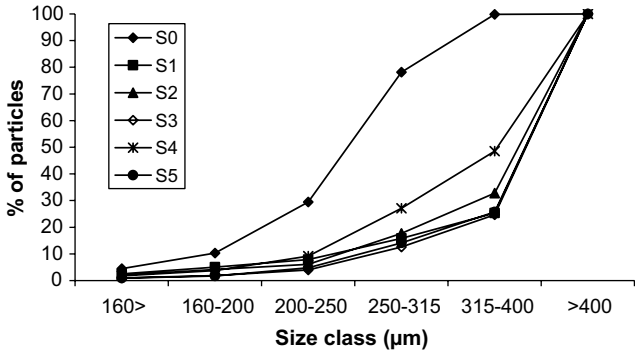

Fig. 7. Cumulative granulometric curve.

tration, the spreading of the liquid on the solid surface is easier (because of the lower viscosity and density), so the covering and the sticking between particles were also higher. In this case however, the processing time was longer and therefore the mechanical stress for these samples was higher, which could cause the abrasion of the products. Hence, an optimum concentration was necessary, where the increase in the aggregates was the lowest (the ratio of the two converse processes was the same). The lowest increase in D50 was detected for sample S4. Thus, this concentration was the most appropriate.

The products were classified according to their particle size. During the fluidization process, the spray-drying of the atomized liquid is inevitable. These particles can be found among the particles smaller than 200 μm. The most appropriate particles measured between 200 and 315 μm; the highest proportion of starting materials is to be found in this range. Thus, the agglomeration is lowest for these particles. The aggregated products were larger than 315 μm.

The proportion of the particles with optimum size was nearly 70% for Arbocel A 300. The ratio of these particles was significantly lower for the samples containing HSA. The highest value was detected for S5, which was ~2 times higher than for the other samples.

3.4. Effect on flowability

It is well-known that the flowability of a sample is determined by the morphological parameters [29]. The flow time of the samples was therefore determined before sieving (Table 3). The flow time was long, with a high deviation, for the particles containing HSA. The best result was detected for S4. This product exhibited the lowest particle size increase. Because of the lower ratio of the irregular sticking of the particles, the shape was better for even flow.

3.5. Effect on content uniformity

The study of the concentration of the active agent revealed that the concentration of HSA was higher in the group containing smaller particles (higher than the calculated value) (Table 4). This can be explained by the spray-drying of the active agent and the abrasion of these spheres from the larger particles (Figs. 3 and 4). The lowest concentration was measured for S3, and in this case the proportion of these particles was also the lowest (Table 4). The formation of these particles was therefore the smallest in this case. Spray-drying is easier for liquids containing a high amount of solid, so the ratio of these particles should be enhanced on increase of the concentration of HSA. However, the larger density and hence the larger viscosity caused a decrease in the sprayability of the liquid. Larger droplets were formed, and thus the evaporation of the liquid took longer. In view of the adverse effects, the optimum value for the concentration of HSA must be found. In this case, 12.5% HSA was optimum. The sticking and the abrasion of the particles were explained previously.

The value for the largest particles could not be exactly evaluated. The determined value was lower than in the other two groups, but it was very variable for the same sample (8.75% was measured for S2, but repetition of this measurement gave >13%, with a high standard deviation (RSD >15%), i.e., the result of the repeated measurements was not comparable). This was influenced by the sampling from agglomerates containing particles with very different particle size.

Table 4
Concentration of HSA (%) in different intermediates

Sample	<200 μm	200–315 μm
S1	16.37 ± 0.94	13.31 ± 0.61
S2	19.10 ± 0.88	12.63 ± 1.25
S3	13.96 ± 0.45	13.49 ± 0.54
S4	18.68 ± 0.83	10.95 ± 1.36
S5	18.90 ± 0.46	14.43 ± 0.28

In the fraction of particles in the interval 200–315 μm, the lowest value was measured for S4 (with the highest RSD). The HSA concentration of the small particles was also high. Accordingly, an uneven distribution of the active agent can be expected. The presence of the active agent could not be evaluated (loss of small particles, or enrichment in the larger particles as a thicker film), because the exact determination of concentration of largest particles was impossible. In spite of its good flowability, S4 was not the best composition because of the uneven distribution of HSA.

4. Discussion

The main aim was to prepare intermediates for the preparation of tablets with different concentrations of HSA. It can be concluded that the yield of the fluidization technique was very good, independently of the concentration of the applied liquid. The process caused an increase in the particle size. The HSA was responsible for the increase, because water did not cause aggregation. The majority of the inert carrier particles were in the range 200–315 μm. During the preparation, the ratio of these particles decreased. The largest proportion was detected for the samples prepared with liquid containing 15% HSA (about 2 times higher than for the second best). In this case, the ratio of the particle size-increasing and destructive processes was the most preferable.

Not only the size, but also the shape of the particles changed, leading to a change in flowability. The concentration of HSA in the fraction containing smaller particles was higher because of the abrasion of the particles and the spray-drying of the HSA. The formation of large aggregates must be eliminated, because the distribution of the active agent is very inhomogeneous.

Finally, it may be stated that this modified layering of powder cellulose with a fluid bed technique can be applied for the production of an intermediate from HSA for the preparation of solid dosage forms. Previously, the appropriate concentration of this protein solution must be optimized, as HSA can act as a binder. Our results indicated that the best value can be reached with liquid containing between 12.5% (most homogeneous distribution of HSA) and 15% HSA (best flowability).

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