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Research paper

Evaluation of the binding effect of human serum albumin on the properties of granules

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ABSTRACT

The main objective of this study was the application of a solution of human serum albumin as a granulating fluid. The properties of the granules formed were evaluated and compared with those when a conventional binder was applied in the same concentration. The powder mixture contained a soluble (mannitol) and an insoluble component (different types of cellulose). The protein solution applied exerted an appropriate aggregating effect if the system contained microcrystalline celluloses. Powdered cellulose was not suitable for the granulation with human serum albumin solution. As compared with the same concentration of the conventionally applied cellulose ethers as binder, the prepared granules exhibited a larger particle size, a significantly better compressibility, a higher breaking hardness and a favourable deformation process. These findings mainly reflect the good adhesive properties of the protein. The best compressibility and mechanical behaviour were attained on the application of the microcrystalline cellulose Vivapur type 105. This favourable behaviour may be connected with the wettability of cellulose. These results suggest that the formulation of tablets may be easier from an active agent in the serum that binds to albumin (e.g. interferon) since the amount of additives (binder) can be reduced.

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]. This may afford a possibility for the direct application of these materials. Proteins have a complex internal structure which helps define their biological activity. Any disruption in the primary (amino acid sequence), secondary (two-dimensional structure), tertiary (folding) or quaternary structure (combination of peptide subunits) can result in the deactivation of a protein. Such disruptions may be caused by even the slightest changes in the environment (or even microenvironment) of the protein. The most likely variables which can affect protein structure and stability are related to the temperature, pH, solvent, other solutes and crystallinity states of the protein [2]. These problems must be considered during the formulation of dosage forms containing proteins.

Since proteins may be very sensitive, the main route of administration is by injection. The injection dosage form, however, has a number of disadvantages, such as low patient compliance, and the possibility of infection and pain during repeated injections [3]. This mode of application can be very inconvenient, and the evaluation of non-invasive routes is therefore very relevant.

Various strategies have been pursued to develop safe and effective oral delivery systems for proteins [4] and to develop sustained and long-acting release delivery systems [5,6].

One possibility is the formulation of buccal preparations. For many drugs, and especially peptides and proteins, the buccal route offers many advantages over conventional modes of delivery, with an improved bioavailability due to the avoidance of degradation in the gastrointestinal tract and hepatic first-pass metabolism [7]. Excellent accessibility, high patient acceptance and compliance, and robustness may be mentioned as the attractive features of buccal administration [8].

The formulation of buccal tablets from biological samples is therefore a reasonable demand. Since the applied proteins are mainly to be found in liquids (e.g. serum), their incorporation into tablets can be complex (e.g. first lyophilization step). This process can cause the degradation of the proteins [9]. To protect a protein from freezing (cryoprotection) and/or dehydration (lyoprotection), a protein stabilizer(s) may be used. On the other hand, overuse of an excipient(s) may eventually destabilize a protein.

The simplest means of tablet making is direct compression [10,11]. In this case, many important parameters (e.g. good flowability and compressibility) must be considered [12]. A problem with direct compression is the relatively high compression force, and hence the higher temperature during the compression (formation of "hot spots" [13–15]).

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The preparation of appropriate intermediates can promote the preparation of tablets. The most widely used method is granulation, in which case separation of the proteins from the liquid is not necessary: may serve as a granulating liquid. It is known from our previous results on human serum albumin (HSA) that it forms a film-like layer, and has an aggregating effect, and there is no degradation during the process at 35–40 °C [16]. Evaluation of the binding effect of the protein is therefore reasonable before choice of the appropriate composition. If the binding effect of the protein solution is acceptable, the amounts of other excipients can be reduced, which is very favourable because of the sensitivity of proteins.

In this study, the granulating effect of HSA solution was evaluated and compared with those of various conventionally used binding cellulose ethers. The influence on different powder mixtures was studied. Mannitol (M: as a conventionally used filler in buccal preparations) and different types of cellulose were applied. A high-shear granulator was used to prepare the samples. The possibilities for binding include the film-forming materials applied in the granulating liquid and also the bridges formed from the soluble M after the recrystallization. The parameters of the granules which are of importance as concerns tablet making were tested.

HSA is a single-chain protein synthesized and secreted from the liver cells. HSA (MW 66,472 Da) is found mainly (~50%) in the plasma, where it maintains the pH and osmotic pressure and plays a major role in transporting a wide range of materials such as metal ions, fatty acids, amino acids, metabolites and many drugs (e.g. interferon) [17–20]. HSA is known to be sensitive to heat, ions, etc. [21–23]. The choice of an appropriate formulation (additives and methods) is therefore very difficult.

2. Experimental

2.1. Materials

Mannitol (M) (Ph.Eur. Hungaropharma Plc., Hungary), different types of microcrystalline cellulose (MCC) (Vivapur 101, 103 and 105, J. Rettenmaier & Söhne GmbH & Co. KG, Germany) and powdered cellulose (PC) (Arbocel P 290, J. Rettenmaier & Söhne GmbH & Co. KG, Germany) were applied in powder mixtures.

The granulating liquids were 4% HSA solution (Trigon Biotechnological Ltd., Hungary), an aqueous solution of hydroxypropylmethylcellulose (HPMC, Pharmacoat 606, Shin-Etsu Chemical Co., Ltd., Japan) and hydroxypropyl-cellulose (HPC, Klucel LF, Hercules Inc., USA) in the same concentration.

Parameters of HSA solution (containing also NaCl, KCl, Na_2HPO_4, KH_2PO_4, etc.) 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.

2.2. Preparation of samples

The samples were prepared in a high-shear granulator (ProCepT 4M8 granulator, ProCepT nv, Belgium).

The powder mixture was prepared from 100 g M and 100 g cellulose. The type of cellulose and the composition of the granulating liquid were varied (Table 1). The amount of water was the same for all the samples. The constant operational parameters were determined in the previous experiments:

- Impeller speed: 750 rpm.
- Chopper speed: 3000 rpm.
- Dosing speed: 5 ml/min.

Table 1
Compositions of samples

Sample	Cellulose	Granulating liquid	Amount of liquid (g)
S1	MCC 101	4% HSA	80
S2	MCC 101	Water	76.8
S3	MCC 101	4% HPMC	80
S4	MCC 101	4% HPC	80
S5	MCC 103	4% HSA	80
S6	MCC 103	Water	76.8
S7	MCC 105	4% HSA	80
S8	MCC 105	Water	76.8
S9	P290	4% HSA	80
S10	P290	Water	76.8

- Spheronization time: 1 min.
- Total granulation time: 17 min.
- Drying: on trays at 40 °C for 2 h.

2.3. Evaluation of samples

The sizes and the size distributions of the samples were evaluated with an analytical sieve (Retsch GmbH, Germany) and a sieving system software (Retsch EasySieve 2.0, Germany). Particles larger than 2 mm were regarded as waste. The yield calculations and all tests were performed after the removal of these particles.

A powder testing apparatus (PTG-1, Pharma Test GmbH, Germany) was used to test the time of the flow of 100 ml of sample. A teflon accessory with an orifice 10 mm in diameter was applied.

The surface tensions of the HSA, HPMC and HPC solutions and the water were measured with a ring method (Krüss GmbH, Germany). A Brookfield LVDV-II viscosimeter with CPE 42 spindle (Brookfield Engineering Laboratories Inc., USA) was used for the determination of the viscosity of the solutions at 25 °C. One milliliter sample was tested at 12 rpm.

Densities (bulk (ρ_0) and tapped (ρ_∞)) were determined with a STAV 2003 Stampfvolumeter (Engelsmann A.G.L., Germany). Carr's index was calculated from these results [24] and three parallel tests were carried out:

$$\text{Carr's index} = \frac{\rho_\infty - \rho_0}{\rho_\infty} \times 100$$

The breaking hardness was tested for granules measuring between 710 and 800 μm. This device contains a special specimen holder and a stamp, and is connected to a computer via an interface; thus, not only can the ultimate deformation force be measured, but the process (force–time and force–displacement curves) can also be followed. If the measured plot (force–time) is parallel to the *x*-axis the deformation is viscoelastic; if the plot

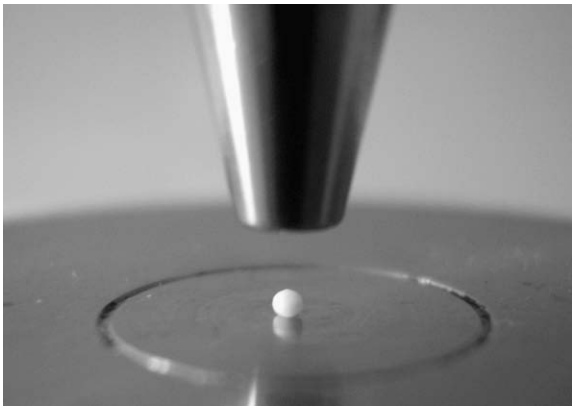

Fig. 1. Sample holder of breaking hardness tester.

rises linearly, the deformation is elastic. The specimen is located horizontally and the stamp moves vertically (Fig. 1). Twenty parallel measurements were performed.

The measuring range was 0–200 N, the speed of the stamp was 20 mm/min, the output was 0–5 V, and the sensitivity was ±0.5% ± 0.1 digit. The sensor was UNICELL force measuring equipment, calibrated with the C9B 20 kN cell.

The Enslin number is a simple semi-quantitative measure of the water uptake of a powder, and is equal to the amount of fluid absorbed by 1 g of the powder (ml/g). An Enslin apparatus with a glass filter and a pipette with 0.01 ml accuracy were used for these experiments. A monolayer of particles took up the maximum quantity of water possible through a filter paper under these conditions. Each powder (0.5 g) was tested; 5 parallel experiments were performed.

Statistica for Windows 7.1 AGA software (StatSoft, Inc. Tulsa, USA) was used for the statistical analysis. The two-sample *T-test* was applied for the comparison of two groups of results. The confidence interval was 95% ($p < 0.05$).

3. Results and discussion

3.1. Particle size

The yields of the samples containing MCC were very good (Table 2). The values for powdered cellulose (S9 and S10) were the lowest because large, very loose flakes were formed in these cases.

The particles of samples produced with the well-known binder were smaller than those of S1. The particles of the samples containing HSA were larger than those of the samples containing the same powder mixture, but prepared only with water (S2, S6, S8 and S10).

The conventionally used binder in the same concentration exhibited lower particle sizes than that of the sample prepared with HSA. The explanation may be the different wetting of the powder mixture and the different binding potency of the binder. The surface tensions of the liquids, which can influence the wetting of the powder mixes, were 42.66 ± 0.62 Nm for HSA solution, 44.92 ± 0.94 Nm for HPMC solution and 40.26 ± 0.43 Nm for HPC solution. There was no relevant difference in this parameter, which was therefore not the main factor responsible for the particle size of the granules. The spreading of the liquid can be different because of variant viscosity (1.5 mPas for HSA solution, 19.1 mPas for HPMC solution and 45.9 mPas for HPC solution). The particle size was lower for granules prepared with viscous solution. The favourable granulating capacity of HSA solution can probably rather be explained by the better binding capacity of HSA solution.

For the evaluation of the effects of the starting materials, different parameters of the MCCs and liquids were considered. The particle sizes, surface tensions, bulk densities and Enslin numbers for water uptake of the different MCCs were determined.

MCCs with higher bulk densities exhibited higher Enslin numbers (Table 3). A higher amount of fibres can bind a higher amount of liquid. When the water uptake was higher, the D50 value for the sample prepared with HSA solution was also higher (Table 2; S1, S5, S7 and S9). The corresponding tendency was opposite for the samples produced with water. The explanation may be that the higher amount of water taken up by MCC cannot act as a binder, since this material is not soluble. The only binder in this composition is the recrystallized M. The proportion of the water available as the solvent of M was therefore lower, and accordingly these granules were smaller. The liquid taken up by the MCC also contained HSA, for the samples were prepared with HSA solution. After drying, this component formed bridges between the particles, and in this case the most important binder was not the recrystallized M, but the HSA. When the amount of granulating fluid taken up was higher, there was a higher possibility of formation of more HSA bridges, and hence the mean size of the samples was higher.

Table 2
Granules size distribution

	Yield (%)	D10 (mm)	D50 (mm)	D90 (mm)
S1	99.21	0.607	0.885	1.284
S2	91.85	0.111	0.608	1.195
S3	87.16	0.114	0.687	1.139
S4	87.06	0.188	0.301	0.904
S5	90.70	0.122	1.054	1.411
S6	84.95	0.068	0.334	0.743
S7	85.53	0.437	0.837	1.194
S8	90.60	0.287	0.696	1.130
S9	57.09	0.248	0.628	1.288
S10	57.45	0.150	0.574	1.272

3.2. Flowability

The properties of the granules were determined. Their flowability and compressibility were better than those of the starting powder mixtures (Table 4). The flow times for the samples with water (S2, S6, S8 and S10) were slightly lower than those for the samples containing binder. This difference was not of importance since these values are very good. The bulk densities of the samples prepared with HSA (S1, S5, S7 and S9) were higher than those of the granules prepared with water (except for S7 and S8, where there was no significant ($p < 0.05$) difference). This parameter was considerably lower for the samples prepared from powdered cellulose (S9 and S10) because they formed large fluffy flakes. The compressibility (Carr index) is another important parameter in tablet making. It was significantly better for the samples containing different binders. This can be explained by the better distribution of the particle size. HSA at this concentration caused a more appreciable increase than the conventional binders.

Table 3
Properties of MCC

Type	Particle size* (μm)	Bulk density* (g/cm^3)	Enslin number (ml/g)
101	50	0.29	2.91 ± 0.05
103	50	0.32	3.03 ± 0.06
105	25	0.23	2.45 ± 0.12

* Data from producer.

Table 4
Properties of granules and powders

Sample	Flow time (s)	Bulk density (g/cm^3)	Carr index (%)	Breaking force (N)
S1	8.0 ± 0.12	0.706 ± 0.011	4.87 ± 0.44	2.64 ± 0.29
S2	7.1 ± 0.15*	0.639 ± 0.006*	11.15 ± 1.39*	2.43 ± 0.55
S3	7.7 ± 0.12	0.670 ± 0.003*	7.69 ± 0.77*	2.44 ± 0.39
S4	6.3 ± 0.12*	0.624 ± 0.001*	7.44 ± 0.44*	1.93 ± 0.36*
S5	8.8 ± 0.6	0.658 ± 0.002	11.79 ± 0.59	2.11 ± 0.35
S6	6.5 ± 0.12**	0.568 ± 0.010**	14.10 ± 0.44**	1.97 ± 0.38
S7	7.9 ± 0.15	0.714 ± 0.007	4.62 ± 0.77	2.70 ± 0.63
S8	7.4 ± 0.12**	0.723 ± 0.006	8.53 ± 0.62**	2.49 ± 0.35
S9	10.2 ± 0.21	0.258 ± 0.005	6.67 ± 0.44	2.36 ± 0.41
S10	9.8 ± 0.25	0.272 ± 0.005**	9.23 ± 3.35	2.28 ± 0.5
MCC101 + M	34.2 ± 6.38	0.399 ± 0.006	24.87 ± 1.94	–
MCC103 + M	31.9 ± 2.2	0.434 ± 0.0004	23.98 ± 2.83	–
MCC105 + M	No flow	0.455 ± 0.012	28.66 ± 2.16	–
PC + M	19.8 ± 0.93	0.434 ± 0.010	25.90 ± 1.60	–

* Significant difference between the sample and S1.
** Significant difference between the samples prepared from the same powder mixture, but with different granulating fluid.

3.3. Mechanical property

The mechanical properties of the granules were better for S1 than for the compositions containing the conventional binders. Higher values were found for the samples containing HAS (S1, S5, S7 and S9), independently of the type of cellulose. The best mechanical properties were those of the sample containing MCC 105 (S7 and S8). The water uptake of this sample was lowest because of the low density. The number of fibres and thus the number of bridges formed may be lower, but the binding may be stronger and accordingly the hardness may be higher. The higher possibility of the binding force of recrystallized M also must be considered. The texture of this sample was therefore the most compacted.

Not only the breaking hardness, but also the deformation process can provide information on the processibility. The breaking curve of S1 (Fig. 2) was very similar to those of the compacted pellets or granules [25]. There were three phases: a short elastic part was followed by a viscoelastic phase, and finally an elastic section up to the breaking point. There were no meaningful irregularities in the curves, which revealed only small deformations caused by the slightly inhomogeneous structure (Fig. 3). S2, prepared with water, exhibited a primarily elastic curve with a short viscoelastic section (Fig. 4). It is well known that air exhibits elastic properties,

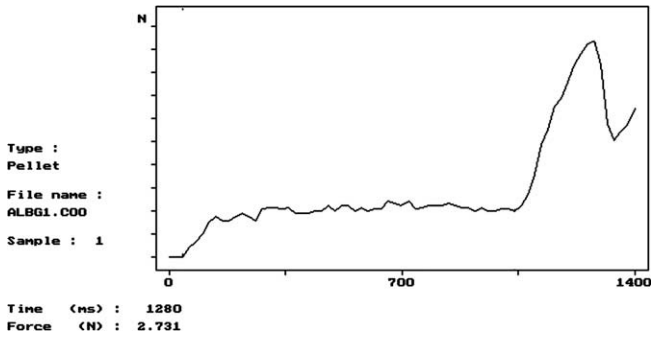

Fig. 2. Breaking hardness curve of sample S1 (granulating fluid: HSA solution).

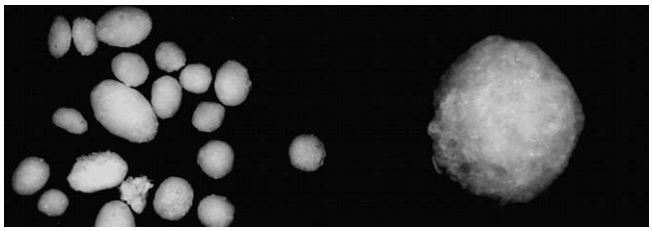

Fig. 3. S1 particles (magnification: 10× (left) and 50× (right)).

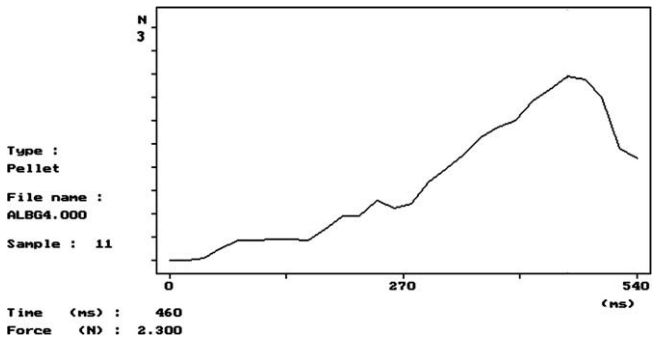

Fig. 4. Breaking hardness curve of sample S2 (granulating fluid: water).

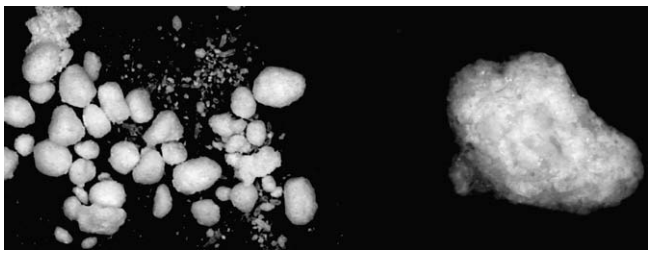

Fig. 5. S2 particles (magnification: 10× (left) and 50× (right)).

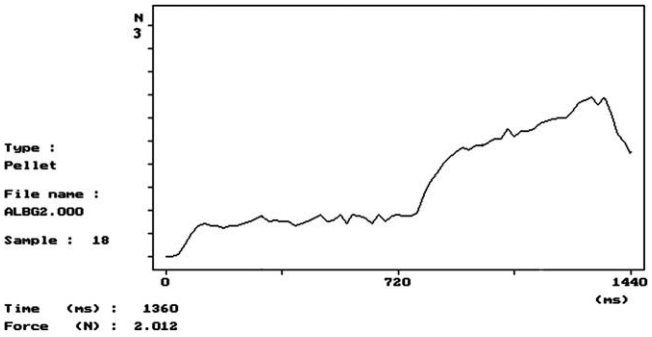

Fig. 6. Breaking hardness curve of sample S3 (granulating fluid: HPMC solution).

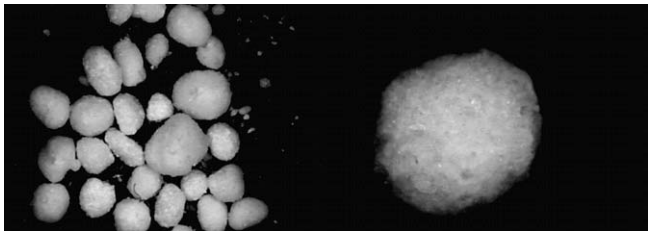

Fig. 7. S3 particles (magnification: 10× (left) and 50× (right)).

and in this case the amount of entrapped air was higher because of the loose structure and irregular shape (Fig. 5). More elastic materials cannot be compressed because of the capping [10]. The curve of S3 (Fig. 6) was better than that of S2, but there were more irregularities than for S1, and the separation of the different phases was also less marked. The shape of these particles was very similar to that of S1 particles (Fig. 7).

4. Conclusions

It can be concluded that the HSA solution had a very good granulating effect when the system contained the studied MCCs. Powdered cellulose was not appropriate for granulation with HSA solution. As compared with the conventionally used binder in the same concentration, the granules formed with HSA displayed a larger particle size, a significantly better compressibility, a higher breaking hardness and a favorable deformation process. The explanation of the advantageous properties is the good adhesive properties of the protein. The different MCCs furnished products with different properties. This was connected with the structure of the particles and their water uptake. According to our results the best compressibility (highest bulk density), and the best mechanical behaviour were detected for materials containing MCC 105 and granulated with HSA solution.

It may be stated that the inclusion of HSA in the granulating fluid can be very useful: pretreatment of this component is not

necessary, the granulating effect is considerable and the amounts of additives can be decreased.

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