MICRONISATION OF HIGH VISCOUS BIOPOLYMERS

C. Reibe[1,2,3]*, Z. Knez[2], E. Weidner[3], P. Alessi[1], A. Kilzer[3].

[1] *Department of Chemical, Environmental and Raw Materials Engineering (D.I.C.A.M.P.) University of Trieste, Piazzale Europa 1, 34127 Trieste, ITALY*
[2]*Laboratory for Separation Processes, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova Ulica 17, 2000 Maribor, SLOVENIA*
[3]*Chair of Process Technology, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, GERMANY*
christian.reibe@vtp.rub.de

1. Motivation

Biopolymers are characterised by their biodegradable behaviour in certain environments. Especially the development of natural biopolymers from renewable resources is gathering increasing interest in scientific as well as in industrial sectors[1]. The advantages can be found not only in the sustainability but also in the "Zero Waste Principle" of these materials, due to the biodegradability[2]. Furthermore, the introduction of renewable polymers helps to save fossil resources[3].

Not only these aspects, but also their physical compatibility[2] increases the interest in them, especially in the medical and the nutritional sector. Since biopolymers may be tailored to specific requirements, the encapsulation of pharmaceutical compounds or flavours in biopolymers would open wide avenues for the production of controlled- release systems.

These days, the synthesis of biopolymers is already quite well understood. However, the subsequent processing still poses problems. The high viscosity, due to the characteristically high molecular mass, results in challenging rheological behaviour. Common processes are using solvents and additives to lower the viscosity and to ease the further handling[4].

Gelatine, as a widely applied biopolymer represents one example of those substances of large interest, but is difficult to process. Especially gelatines with high molecular mass are difficult or almost impossible to be pulverized.

In this research an integrated spraying, drying and pulverization process for gelatine is presented.

2. Gelatine

Type A and type B gelatine was provided by *Gelita AG, Eberbach, Germany*. Each type exhibiting a gel-strength of 100, respectively 200 Bloom.

2.1 *Properties*

Gelatine is used as encapsulation material in a wide range of applications. Gelatine is extracted from collagen-containing resources (such as pigskin and cattle split) by thermal or enzymatic hydrolysis. It is an albuminoid based on a polypeptide-chain, comprising around 1000 amino acids. The most important amino acids in gelatine are glycine (27 %), proline and hydroxyproline (25 %). The residual part is shared by another 17 amino acids. Three of the polypeptide chains, the so called "α-chains" form a right handed triple helix coil. Structurally typical gelatine sequences are repeating glycine x-y triplets, shown in figure 1. While x and y are frequently proline and hydroxyproline[4].

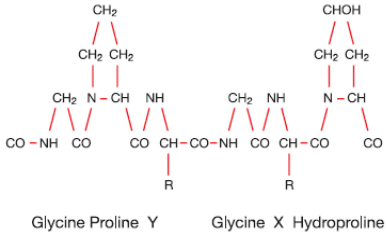

Fig. 1: Typical gelatine sequence [5]

The molecular mass of gelatine is typically

between 13.5 kDa and 500 kDa. and depends on the production procedure. Gelatine is derived from an extraction process, using hot water as solvent. The extraction temperature is increased from 55 °C at the beginning up to 100 °C at maximum. It is the leading factor for the gelatines' viscosity. The lower the extraction temperature, the higher is the gel-strength[5].

2.2 Characterisation

Gel strength and viscosity are quality-defining parameters. The gel-strength is investigated with a defined standard (either the British Standard (B.S.) or the standard of the American Organisation of Analytical chemistry (AOAC))[6]. Due to the fact that the apparatus for measuring the weight was invented by Bloom[7], the gel-strength is called the Bloom-number and expressed in Bloom-gram. High- Bloom gelatines have Bloom numbers above 200, while low-Bloom means gelatine with less than 100 Bloom-gram. The Bloom number is equivalent to the viscosity. The higher the Bloom number, the higher is the viscosity.

2.3 Usage

Gelatine is not only used in the pharmaceutical industry as encapsulating substance but also in food industry e.g. for the clarification of juice and beer and everywhere, where gelling agents, stabilisers, binding agents, emulsifying agents, foaming agents and thickening agents are needed. Since gelatine does neither comprise fat nor carbon hydrates nor cholesterol nor purine nor any preservatives it is a common, inoffensive additive to many applications[8].

3. State of the art

3.1 Gelatine production

Gelatine usually is produced by hydrolysis of animal collagen. The extract consists of a 5 % aqueous gelatine solution, which is concentrated in vacuum dryers. Those concentrates are pressed to noodles, to be dried in conveyor driers to a residual water content of around 10 %. For the drying, filtered and microbiologically clean air is used. Due to the relatively low melting point of gelatine, this drying is carried out at temperatures between 30 °C and 60 °C. Milling- and sieving- processes are applied for powder generation, classification and standardisation of the final product. Since huge amounts of water are used for the extraction process, the drying process requires huge amounts of energy. Additionally, standardisation and classification processes are energy consuming.

3.2 Gelatine pulverisation

Beside milling and sieving, spray drying is well known to be an adequate process for drying and pulverisation. These days it is the favoured process for preparing fine powders from solutions, by evaporating the solvent. The main idea of spray drying is to transform liquid products to solid powders. Figure 2 schematically shows a common spray dryer.

The liquid is atomised through a nozzle into a spray tower. At the same time hot air is injected. Common temperatures of the hot air stream are between 80 °C and 600 °C. The water is evaporated by the hot air and the product falls in powderous form to the spray towers' bottom. The separation of the finer particles from the hot humid air may be carried out in a cyclone.

Depending on the parameters of the process, the properties of the resulting powders (e.g. particle morphology, particle size and residual humidity) can be adjusted.

The spray drying of gelatine, exhibiting a high molecular weight, is

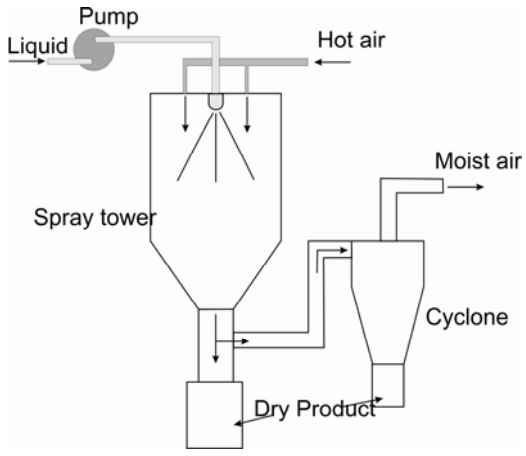

Fig. 2: Spray drying scheme

problematic due to the gelatines' high viscosity. The occurring problem is described as the forming of fibres and filaments, which leads to a blockage of the nozzle[8]. Therefore, it is common to spray dry aqueous solutions with either "low Bloom gelatine" (0-80 Bloom) or completely hydrolysed gelatine (0-Bloom) or aqueous solutions with very low amounts (5 wt-%) of high molecular gelatine.

Another possibility to prevent the blockage of the nozzle is to apply additives like softeners (e.g. glycerol), dusting agents (e.g. carbon hydrates) or viscosity-reducing agents like edible oils. Most commonly, the amount of these substances is more than four times the amount of gelatine, used.

We propose a process, where pure, high bloom gelatine can be spray-dried and powderised without applying additives.

4. Experimental

4.1 Process-description

The process' main principle is to atomise an aqueous gelatine solution, mixed with tempered carbon dioxide (CO_2), through a nozzle (8) into a spraying chamber (9). Therefore an aqueous gelatine solution is preheated in a reservoir (1),. For the monitoring of the gelatine-mass flow, the reservoir is stored on a scale (2). A three-head diaphragm-pump (3) delivers the gelatine- sol to a T-piece, where it is mixed with the CO_2, delivered from a tank (4) and compressed also by a three-head diaphragm pump (5). A Coriolis- flowmeter (6) monitors the mass flow of the CO_2, which is heated downstream in a heat exchanger (7). After the expansion in the spraying chamber the excess fluid with the finer particles is vented through a cyclone by means of a blower(12). In the cyclone (10) the fines are separated and collected in a bin (11). For experiment performance, the spray tower is preheated by flushing it with hot CO_2. As soon as the required temperature is reached, the gelatine-flow is started. The process is monitored by a computer that also saves the process-data. The leading parameters for successful experiments are the temperature of the starting solution, the temperature of the CO_2, the temperature in the spray tower, the ratios between the $\underline{C}O_2$ stream versus the aqueous- Gelatine- solution- stream (from now on abbreviated by "*RCG")* and the pre-expansion pressure.

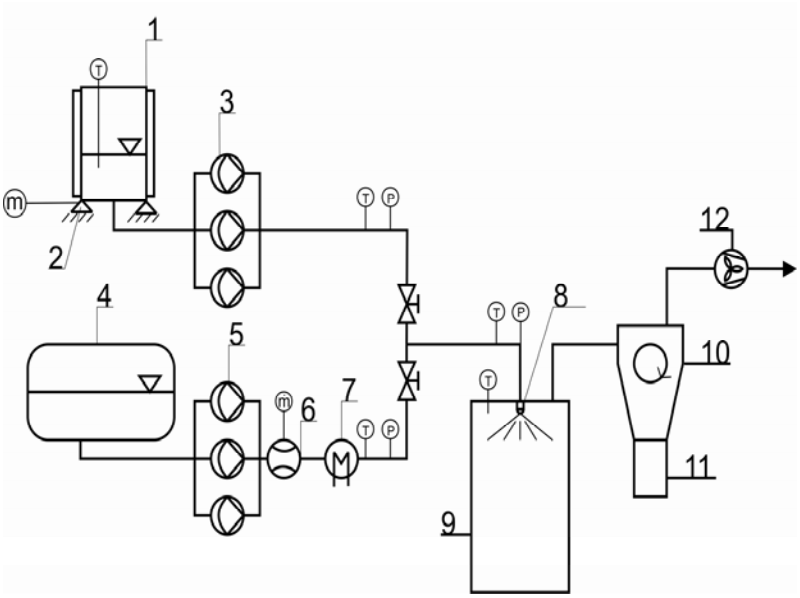

Fig. 3: Micronisation plant

5. Analytics

The resulting powders were analysed by several methods. The characterisation was carried out to obtain relevant data for possible applications.

5.1 Moisture content

The determination of the residual moisture content was done, according to the standard method *NF V 59-003*[9]. Samples of the spray- dried powder were kept at 105 °C ± 1 °C for 16 hours. After removing them from the oven, they were put into a desiccator for cooling down to room temperature. The loss in weight was determined gravimetrically, being the evaporated residual water content. The method was found to be applicable, due to investigations on the time needed to reach a stable weight of the sample. Each sample was investigated three times for statistical consistency.

5.2 Particle size

The powders were analysed with a particle size analyser from *Malvern Instruments (Malvern Mastersizer 2000)*. Its method is based on laser diffraction according to the Fraunhofer- Ansatz. The powders were investigated, by using a dry disperser.

5.3 Morphology

The morphology of the gelatine powder was determined by using a SEM. The SEM- pictures of the gelatine particles were taken at the SEM of the Ruhr-University of Bochum, at the Institute for Geology, Mineralogy and Geophysics. The used SEM was a Gemini 1530 by the company LEO.

5.4 Molecular weight

Molecular weights were kindly investigated by Gelita, using gel permeation chromatography (GPC). The GPC- is a liquid chromatography method. The separation takes place, only due to the different hydrodynamic volume of the molecules in solution. The information obtained by the GPC is the median molecular weight of the investigated substance.

6. Results and Discussion

6.1 Experiments' parameters

Several experiments were carried out with varying gelatine concentrations, different temperatures in the spray- tower and different RCGs. Figure 4 and Figure 5 are presenting parameters used for the micronisation process. These parameters were applied in experiments in order to powderise 200 Bloom gelatine of type B, while the pre-expansion pressures were in the range from 75 bar to 85 bar.

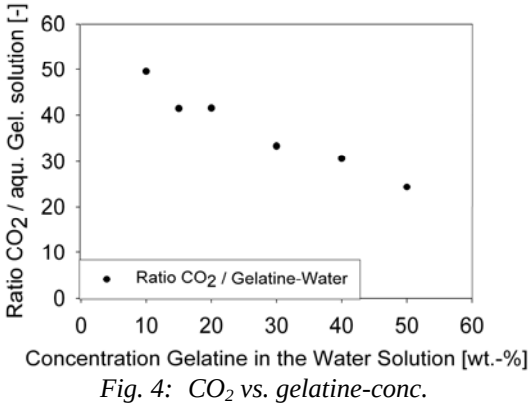

Fig. 4: CO_2 vs. gelatine-conc.

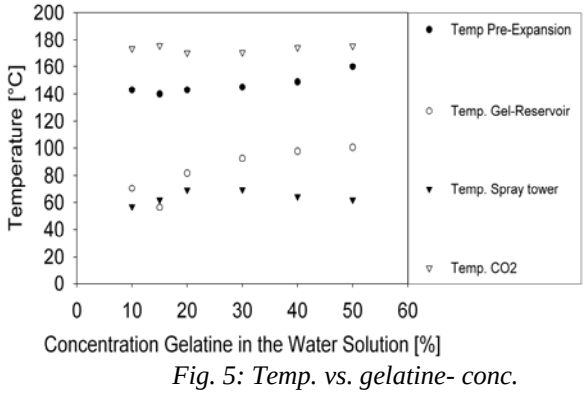

Fig. 5: Temp. vs. gelatine- conc.

Solutions with gelatine contents between 10 wt.-% and 50 wt.-% of dry gelatine matter were micronised. Figure 4 points out the influence of the ratio between CO_2 and the aqueous gelatine solution on the gelatine concentration, regarding the success in obtaining a dry product. For drying and micronising an aqueous gelatine solution, containing only 15 wt-% of gelatine, the

RCG has to be around 40, while a solution, containing 50 % gelatine in the aqueous solution only requires a RCG of around 25. Increasing the gelatine amount in the solution is logically implementing a lower water-content. The observed behaviour is obviously based on the fact, that less water has to be removed and therefore less CO_2 is needed. Figure 5 shows the different process temperatures of the same experiments. It can be seen that at higher gelatine concentrations in the aqueous solution, higher temperatures are needed in the gelatine reservoir. A 15 wt.-% gelatine solution requires a temperature less than 60°C for being pumpable with the installed pump, while the temperature of the 50 wt.-% solution needed to be close to 100°C. This can be explained by the increasing viscosity of higher concentrations of gelatine in aqueous solutions. The other temperatures could be kept almost constant with increasing gelatine concentration. Only due to the reduced amount of CO_2, used for the drying of higher concentrated solutions, the CO_2-temperature has to be increased slightly to keep the required temperature in the spray tower.

6.2 Moisture content

The residual moisture content was determined by the standard, mentioned above. In each case, powder was obtained; the moisture content was between 8 % and 13 %. This range of residual water contents is known to be the water content, exhibited by gelatine in the equilibrium with the atmosphere. Water content in gelatine below 16 % results in a stable product, where no microbiological degradation occurs.

6.3 Size

The mean size for most of the micronised powders was found to be around 300µm. As can be seen from Figure 6, the size of the different types of gelatine, sprayed at approximately the same conditions, do not show a remarkable effect due to the different types of gelatine.

Powders, no matter whether 100 Bloom or 200 Bloom, produced from type A gelatine, as well as from type B gelatine, exhibited mean particle sizes of around 300 µm.

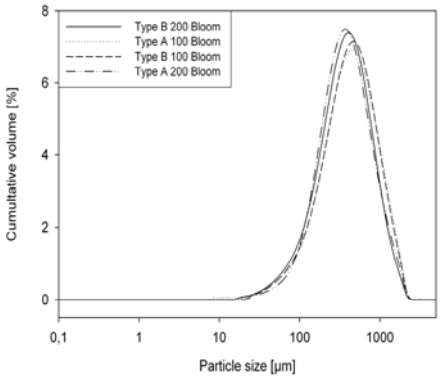

Fig. 6: Particle size distribution (different types of gelatine at similar process parameters)

6.4 Morphology

Figure 7 shows representing SEM-pictures of produced particles from two different resolutions. The picture on the left hand side points out typically fibres as well as knots and undefined structures. In the second picture, the resolution factor was increased by the factor 10. Neither the particle's shape, nor its texture can be categorised.

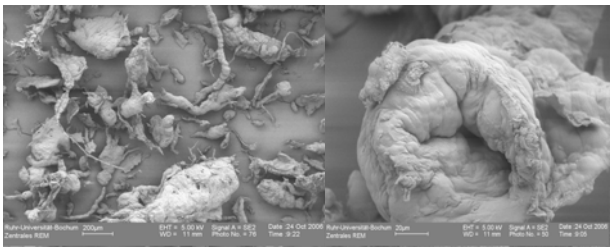

Fig. 7: SEM-Pictures of gelatine powder's particles

6.5 Molecular weight distribution

Figure 7 and Figure 8 present the molecular weight distribution of one of the used feedstock gelatines (type B 200 Bloom) and the molecular weight distribution of a micronised powder.

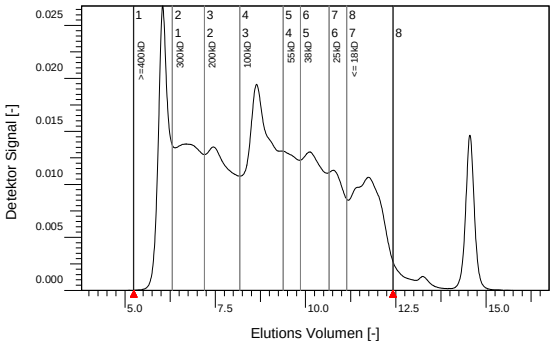

Fig. 8: Feedstock gelatine

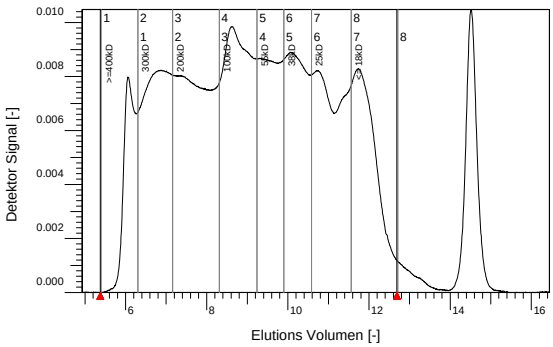

Fig. 9: Gelatine powder

Figure 8 shows the distribution of 200 Bloom gelatine of type B. The mean molecular weight is 156 kD. The mean molecular weight of the powderised gelatine is around 124 kD. The curve indicates the detector signal versus the elution's' volume. The lower the Elution's volume, the higher is the molecular weight. The higher the detector signal, the larger is the amount (in percentage) of long- chain molecules in the indicated range. Eye catcher in Figure 8 is the large peak in the field of long- chain molecules of around 400 kD. Comparing it with the gelatine powder's distribution curve in Figure 9, it can be seen, that this peak is not occurring anymore, while the rest of the curve does not show a significant change in the molecular length composition due to the processing.

7. Conclusion

A process was found for the micronisation of high molecular biopolymers, gelatine in particular. By using supercritical carbon dioxide for atomizing an aqueous gelatine solution, dry, fine and microbiologically stable gelatine powders were produced. Neither the gelatine types, nor its Bloom- numbers showed remarkable influence on the particle size. High viscous solutions, either due to the high gelatine concentration, or due to the higher molecular weight of the solution, require higher temperatures to enable a satisfying process. Furthermore it was shown, that the process just has a slight effect on fractionising the long chain molecules. The resulting powder still exhibits high molecular weight.

8. Acknowledgements

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