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Desolvation process and surface characteristics of HSA-nanoparticles

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Abstract

The objective of the present study was to characterise and optimise the desolvation process of human serum albumin (HSA) for the preparation of nanoparticles. Following the desolvation of the protein, the resulting nanoparticles were stabilised by the addition of varying amounts of glutaraldehyde. The particle size and the number of available amino groups on the surface of the nanoparticles were determined. The results indicated that the particle size depended mainly on the amount of desolvating agent added, but not on the amount of cross-linker. Increasing volumes of glutaraldehyde reduced the number of amino groups on the surface of HSA nanoparticles.

Keywords: Nanoparticles; Human serum albumin (HSA); Surface characterisation; Desolvation procedure; Amino group determination

The major advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution (Kreuter, 1983) as well as the enhancement of the cellular uptake (Schäfer et al., 1992). Among these colloidal systems those which are based on proteins may be very promising, since they are biodegradable and non-antigenic (Rubino et al., 1993), relatively easy to prepare and their size distribution can be monitored (MacAdam et al., 1997). Because of the defined primary structure of proteins the protein-based nanoparticles offer various possibilities for surface modification and covalent drug attachment.

The objective of the present study was to evaluate the desolvation of human serum albumin (HSA) for the preparation of nanoparticles by investigation of particle size and the percentage of protein still dissolved in the reaction mixture. Further investigations were focused on the influence of various glutaraldehyde concentrations on the number of available amino groups at the surface of HSA nanoparticles what may also be a measure for the degree of cross-linking.

For evaluation of the desolvation process, the samples were prepared as follows: under constant stirring aliquots of either 0.25 or 0.1 ml ethanol (total 8.0 ml) were added dropwise to 2.0 ml of a 10% aqueous HSA solution. After each desolvation step, aliquots of 0.05 ml were taken from the samples, and cross-linked with glutaraldehyde.

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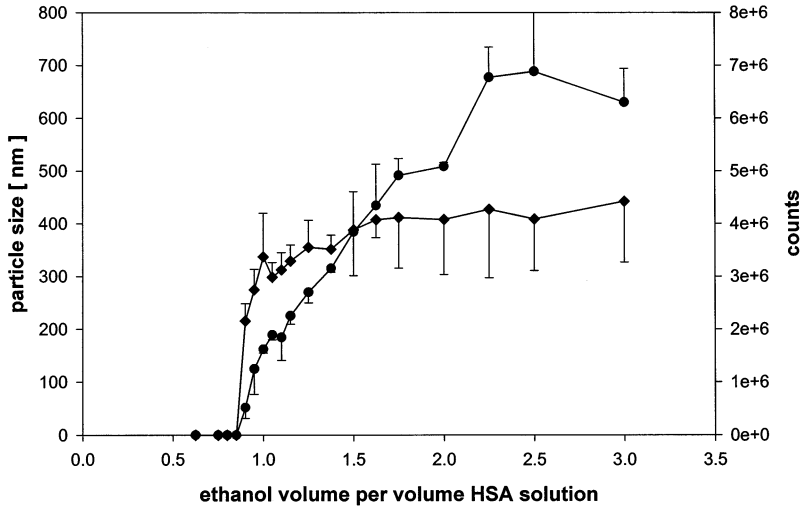

Fig. 1. Human serum albumin (HSA) nanoparticles prepared by desolvation process: particle size (—◆—) and light intensity counts in the photon correlation spectroscopy (PCS) measurement (—●—) in correlation to the amount of ethanol added during the desolvation procedure (mean ± S.D.; $n = 3$).

For the evaluation of the desolvation process, the particle size and the percentage of dissolved protein were determined. The particle size as well as the cumulative light intensity counts of the samples were determined by photon correlation spectroscopy (PCS). The light intensity pulses depend on the particle concentration in the test tube as well as on the particle size in that the countrate increases with the size. Up to addition of a 1.5-fold volume of ethanol relative to the volume of the initial HSA solution, the particle size increased significantly (Fig. 1). The further addition of ethanol caused no changes in particle size. However, the increase in the number of lightintensity counts up to the 2.5-fold ethanol volume can be taken as an indication that the desolvationprocess was not completed and that with the addition of further ethanol the number of particles

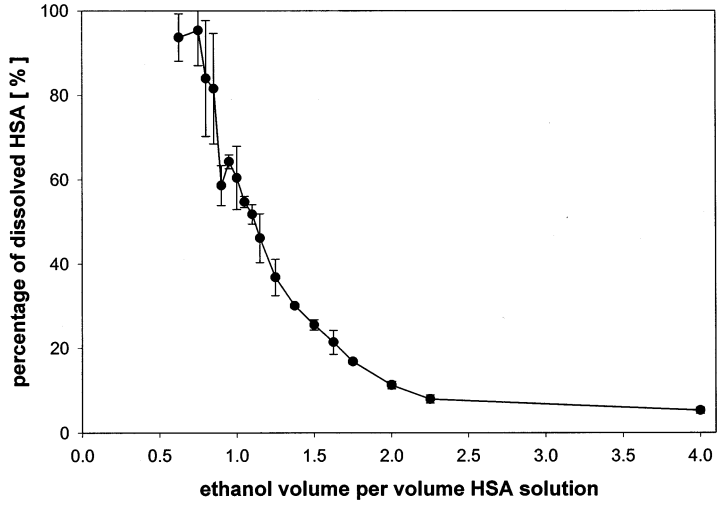

Fig. 2. Human serum albumin (HSA) nanoparticles prepared by desolvation process: Percentage of dissolved HSA in the supernatant of the nanoparticles in correlation to the amount of ethanol added during the desolvation procedure (mean ± S.D.; $n = 3$).

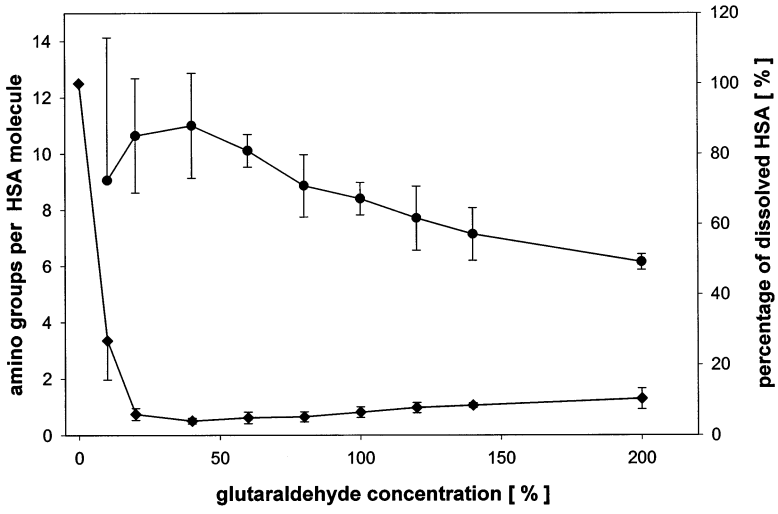

Fig. 3. Stabilisation of human serum albumin (HSA) nanoparticles with different amounts of glutaraldehyde: amino group content (—●—) and percentage of dissolved HSA in the supernatant of the nanoparticles (—◆—) in correlation to the amount of glutaraldehyde added (mean ± S.D.; $n = 3$).

increased. This conclusion was supported by the results of the determination of dissolved HSA in the dispersion medium using a standard BCA protein assay (Fig. 2). Therefore the desolvation process can be divided into two parts: a first part where an increase in desolvating agent leads to an increase in particle size and a second part above a 1.5-fold ethanol volume addition where the particle size remains constant but the particle concentration is still increasing.

Further investigations were focused on the influence of the cross-linker on the number of available amino groups on the surface of HSA-nanoparticles. They were prepared by a desolvation technique previously described by Marty et al. (1978). Different amounts of aldehyde concentrations ranging between 0 and 200% of the theoretic amount that is necessary for the quantitative cross-linking of the 59 amino groups in the HSA molecule (Carter and Ho, 1994) were added to the desolvated HSA nanoparticles.

The determination of amino groups was performed using the reaction of 2,4,6-trinitrobenzenesulfonic acid (TNBS) with free amino groups (Habeeb, 1966). After the reaction the nanoparticles were separated from the supernatant by centrifugation and the supernatant was assayed at 349 nm for unreacted TNBS. The results (Fig. 3) revealed a significant decrease of the number of available amino groups with increasing amounts of glutaraldehyde. This decrease also indicated a progressive degree of cross-linking as already reported by Lin et al. (1994) for modified HSA nanospheres. The unexpectedly low number of amino groups on the nanoparticle surface at low cross-linker concentrations was probably caused by soluble (non-particulate) TNBS-HSA conjugates that increased the absorption at 349 nm in the supernatant after centrifugation of the nanoparticles. This conclusion was supported by the quantitation of the percentage of dissolved HSA in the supernatant of the nanoparticles, where about 30% of the desolvated HSA redissolved again after dilution of the nanoparticles with water in the 10% glutaraldehyde samples. Consequently these particles were not sufficiently stabilised by cross-linking.

The determination of the particle size of purified and unpurified HSA nanoparticles cross-linked with 10 or 20% glutaraldehyde also revealed a slight instability for nanoparticles (Fig. 4). Above 20% glutaraldehyde the different aldehyde concentrations used for cross-linking appeared to have no significant effect on the particle size. Purification of the particulate system by washing four times with water slightly increased the average diameter of the particles. This may be due to swelling of the protein

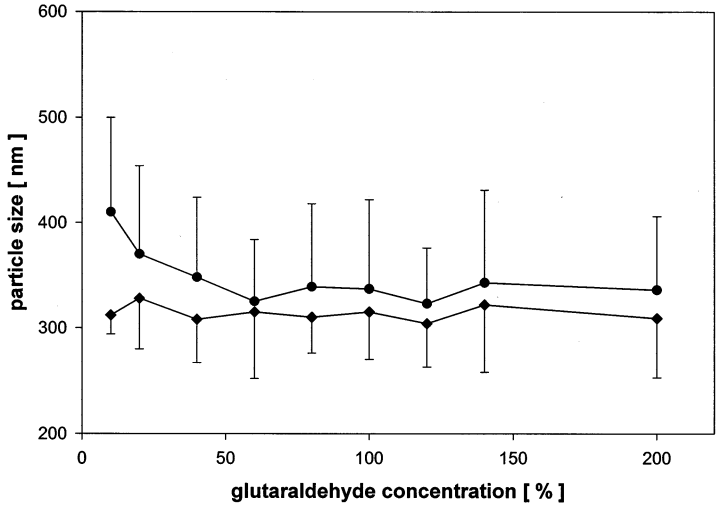

Fig. 4. Stabilisation of human serum albumin (HSA) nanoparticles with different amounts of glutaraldehyde: particle size of purified (—●—) and unpurified (—◆—) nanoparticles in correlation to the amount of glutaraldehyde added (mean ± S.D.; $n = 3$).

matrix of the particles after removal of the desolvating agent ethanol.

The lowest required glutaraldehyde concentration for the production of stable nanoparticles appeared to be about 40% which is in agreement with earlier results of Roser and Kissel (1993).

The evaluation of the desolvation process of HSA with ethanol as desolvation agent revealed that in the first part of the desolvation process the particle size is monitored by the amount of added desolvation agent, in the second part, however, only the particle concentration is influenced by additionally added ethanol but not the particle size. In order to prepare a drug carrier system by covalent attachment of the drug to the colloidal system, the amount of available amino groups on the particle surface is one of the important parameters. As expected, the number of available amino groups decreased significantly with increasing amounts of added glutaraldehyde whereas the particle size remained unaffected. A cross-linker concentration of about 40% glutaraldehyde was found to be necessary for the production of stable HSA-nanoparticles.

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