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In situ coating—An approach for particle modification and encapsulation of proteins during spray-drying

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Abstract

In this paper, we present a method for *in situ* coating of individual protein particles in a respirable size. The aim of the coating was to influence the particle/powder properties, and to reduce or prevent surface-induced conformational changes of the protein, during spray-drying, which was the method used for simultaneously preparing and coating particles. The investigated formulations included bovine serum albumin (BSA), trehalose and either of the two non-ionic polymers, hydroxypropyl methylcellulose (HPMC) and poly(ethylene oxide)–poly(propylene oxide) triblock co-polymer (Poloxamer 188). Complete protein coating as measured by electron spectroscopy for chemical analysis (ESCA) was achieved at a polymer concentration of approximately 1% of the total solids weight, and could be predicted from the dynamic surface tension at the air/water interface, as measured by the pendant drop method. Further, particle properties such as: size, dissolution time, powder flowability, and apparent particle density, as measured by gas pycnometry, were affected by the type and concentration of the polymer. In addition, the particle surface morphology could possibly be correlated to the surface elasticity of the droplet surface during drying. Moreover, an extensive investigation (Fourier transform infrared spectroscopy, circular dichroism and size exclusion chromatography) of the structural effects of protein encapsulated in a polymeric coating suggested that *in situ* coating provide particulate formulations with preserved native conformation and with a high stability during rehydration.

Keywords: Polymeric particle coating; Spray-drying; Competitive surface adsorption; Dynamic surface tension; Surface morphology

1. Introduction

Particle coating, to alter surface properties, to add functionality or for chemical or physical protection of sensitive ingredients, is important in many applications. In particular, there is an industrial need for methods suitable for coating of small particles (<10 μm). Coatings are provided to improve stability, dispersability, flowability or release properties of powders (Gibbs et al., 1999). Coating methods presented in the literature include the use of, *e.g.* supercritical fluids (Jung and Perrut, 2001) or plasma (Bayer et al., 1998) as complement to conventional fluidized bed coating (Jono et al., 2000). Spray-drying has also been used for coating or surface-modification of spray-dried water-soluble small molecules (Wan et al., 1991; Columbano et al., 2003), where the presence of a surface coating by the excipient has been determined by indirect methods. Particles can also be coated by particles, using a mechanical force, *i.e.* dry coating (Pfeffer et al., 2001). Frequently methods claim improved surface coverage and less agglomeration of primary particles. For example, agglomeration of primary particles of corn starch during coating with hydroxypropyl cellulose was minimized by a novel rotating fluidized bed coater (Watano et al., 2004), but still an increase in the particle size from 15 to 25 μm (Watano et al., 2004) would be considered unacceptable for many applications, *e.g.* inhalation particles. Despite progress and innovation in the field of microencapsulation (Gibbs et al., 1999) and surface nanotechnology (Caruso, 2001) literature is still scarce of examples representing coating methods suitable for fine pharmaceutical particles. Not only the size, but also the type of core materials and coatings are unacceptable or inappropriate for pharmaceutics.

In this paper, we have utilized that competitive surface adsorption occurs between surface-active substances in liquid formulations in order to encapsulate and protect a sensitive protein formulation, as well as modifying the powder properties. Competitive adsorption is a common feature of many systems containing a mixture of surface-active species, *e.g.* proteins.

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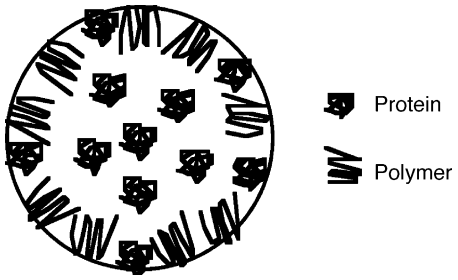

Fig. 1. Schematic illustration of the formulation concept *in situ* coating.

Surface competition during spray-drying implies adsorption of surface-active components to the air/liquid interface of drying droplets. In contrast to steady-state adsorption the time-scale of relevance in spray-drying is restricted. The average lifetime of droplet surfaces, in a laboratory dryer was estimated to be 0.1–1 ms (Elversson and Millqvist-Fureby, 2005b). Consequently, transport and attachment to the interface is important for the competitiveness during spray-drying. At the surface, a protein or polymer that can rapidly rearrange and expose non-polar regions towards the air phase would be expected to be favored.

Several studies establish the fact that the composition of the droplet surface is preserved during spray-drying (Fäldt and Bergenståhl, 1994; Millqvist-Fureby et al., 1999; Landström et al., 2000; Adler et al., 2000; Elversson and Millqvist-Fureby, 2005a). For example, in mixtures of BSA or sodium caseinate and lactose, protein is accumulated at the air/water interface during spraying and thus appears on the powder surface (Fäldt and Bergenståhl, 1994). In contrast, neither of the components is preferentially accumulated in a mixture of glycine and lactose and thus, the surface composition of the powder reflects the composition of the spray solution (Fäldt and Bergenståhl, 1994). It is hence possible to utilize surface competition during spray-drying in order to create desired powder properties, *e.g.* wettability, dissolution (Elofsson and Millqvist-Fureby, 2000) and surface morphology (Elversson and Millqvist-Fureby, 2005a).

Proteins tend to denature when exposed to an air/water interface, since the protein is frequently unfolded. Considering the large surface area in a spray, the potential for surface-induced denaturation is substantial (Mumenthaler et al., 1994; Maa et al., 1998; Millqvist-Fureby et al., 1999), in particular at a low protein load. Possibly, addition of a polymeric coating to protein formulations prepared by spray-drying can enhance protein stability by preventing/reducing protein–surface interactions. This has been observed for protein formulations with addition of low-molecular weight surfactants such as polyoxy ethylene sorbitan esters, Polysorbate 20 (Maa et al., 1998) and Polysorbate 80 (Mumenthaler et al., 1994; Broadhead et al., 1994; Millqvist-Fureby et al., 1999), or sodium dodecyl sulphate (Adler et al., 2000). Provided the coating material, added to the protein formulation, is the most efficiently adsorbing component it will form a protective layer at the droplet surface minimizing the hydrophobic interaction between protein and air/liquid interface during drying (Fig. 1). It has previously been observed that surface-active polymers tend to provide an efficient coating of spray-dried particles at higher polymer concentrations than studied here (Millqvist-Fureby et al., 2000; Elversson and Millqvist-Fureby, 2005a). This formulation concept we call *in situ* coating since both coating and particle formation occur simultaneously, during spray-drying.

Consequently, *in situ* coating is a formulation concept for stabilization of protein formulations during spray-drying but this method can also be used for pure coating reasons, *e.g.* modified release or oxidative protection. Since spray-drying enables formation and coating of individual particles in a single step, concerns on adhesion or wetting capability of coatings, coating homogeneity and agglomeration of primary particles are minimized. The objective of this study was to influence specific particle properties and to reduce or prevent surface-induced conformational changes of protein during spray-drying by *in situ* coating.

2. Materials & methods

2.1. Materials

Solutions with different concentrations of non-ionic polymer were prepared from stock solution of BSA (Cohn fraction V, >96% purity, M_w ~67 kDa, Sigma Chemical Co., St. Louis, MO), D(+)-trehalose dihydrate (Fluka Chemie GmbH, Buchs, Switzerland) and HPMC (M_w ~10 kDa, Aldrich, Milwaukee, WI) or Poloxamer 188 (M_w ~7680–9510 Da, PEO_{80}-$PPO_{27}PEO_{80}$, Uniqema, Gouda, The Netherlands) to obtain a final concentration of 0.5% (w/w) BSA, 9.5% (w/w) trehalose and 0, 0.01, 0.1 and 1% (w/w) polymer. A 10 mM sodium phosphate buffer (pH 7.0) prepared from ultra-purified water (MilliQ, Millipore Systems, 18.2 MΩS resistivity), $HNa_2PO_4{\cdot}12H_2O$ (Fluka Chemie GmbH, Buchs, Switzerland) and $NaH_2PO_4{\cdot}H_2O$ (Merck Eurolab, Stockholm, Sweden) was used as solvent. Fluoroscein isothiocyanate (FITC) labeled BSA (Sigma Chemical Co., St. Louis, MO) was used for CLSM imaging.

All glassware was cleaned thoroughly by a surfactant-free wash solution (Deconex 20NS, Borer Chemie AG, Zuchwil, Switzerland) and rinsed thoroughly with hot and cold water.

2.2. Spray-drying

Hydroxypropyl methylcellulose (HPMC) was dispersed in cold buffer (10 mM phosphate, pH 7.0) under vigorous stirring and once dissolved the stock solution was kept for more than 24 h before use to allow complete hydration and swelling of the polymer. Particles were prepared in a co-current lab-scale spray-dryer (construction of the Institute for Surface Chemistry) (Elversson et al., 2003). The inlet air temperature was 180 °C and the outlet temperature was kept at 70 °C. Liquid feed was 5 ml/min, atomization airflow was 28 l/min and the drying airflow was 0.8 m^3/min. The particles were separated from the drying air by a cyclone. Samples were stored in a desiccators at room temperature and <20% RH until analysis.

Table 1
Temperature cycle for DSC analysis of spray-dried powders

Segment	Cycle	Temperature cycle (°C)	Rate (°C/min)	Residence time (min)
1	Heating	25–140	10	–
2	Cooling	140–90	20	–
3	Isothermal	90	–	1
4	Heating	90–160	10	–
5	Isothermal	160	–	1
6	Cooling	160–140	20	–
7	Heating	140–240	10	–
8	Isothermal	240	–	1
9	Cooling	240–25	50	–

The nitrogen flow was 40 ml/min.

2.3. Dynamic surface tensiometry (pendant drop)

Surface tensions at the air/water interface of protein and polymer solutions were measured by the pendant drop technique (First Ten Ångstroms AccuSoft, Version 1.961B, Portsmouth, VA). In this technique, the surface tension is calculated from the size and shape of a droplet hanging from a tip of a syringe with a blunt-end metal or Teflon-coated needle (Hamilton Microlitre™ Syringes, Hamilton Bonaduz AG, Switzerland). The tensiometer was calibrated with 95% ethanol (23.1 mN/m) and MilliQ-water (72.3 mN/m), at room temperature (23 °C). The sample solutions were monitored for approximately 14 s. The first data point was collected approximately 0.07 s after formation of the drop.

2.4. Scanning electron microscopy (SEM)

The particle shape and the surface morphology were examined with scanning electron microscopy (SEM) (XL30TMP, Fei Company, Hillsboro, ON) in high vacuum mode. The acceleration voltage was typically 25 kV. Powder was sprinkled on a SEM-stub covered by adhesive carbon tape and sputter coated (SCD 050, Balzers Union AG, Balzers, Lichtenstein) with Au of 640 Å thickness (180 s).

2.5. Confocal laser-scanning microscopy (CLSM)

Coated and uncoated particles were imaged in a confocal laser-scanning microscope (Zeiss LSM 510 Meta, Germany) with a 63×/1.3 oil objective. The 488 nm line of an Ar laser was used to image FITC-labeled BSA, using a LP 505 nm emission filter. The pinhole was set to 0.7 μm. A higher effect of the laser resulted in photo bleaching of the particles, which confirmed that the detected signal was from fluorescence and not reflected light.

2.6. Electron spectroscopy for chemical analysis (ESCA)

The elemental surface composition of spray-dried particles was assessed by ESCA (AXIS HS photoelectron spectrometer, Kratos Analytical, UK). The instrument used a monochromatic Al Kα X-ray light source. Powder was filled into DSC cribles, and placed under vacuum overnight. The analysis area was approximately 1 mm^2 and the depth of analysis was less than 100 Å. Analysis was performed in triplicates at different spots within a total area of 20 mm^2.

The surface composition of the powder was estimated from the relative amounts of carbon, oxygen and nitrogen in the pure ingredients (BSA and excipients) and in the spray-dried samples (Fäldt et al., 1993). The percentage surface coverage was calculated according to a matrix model described in detail elsewhere (Elversson and Millqvist-Fureby, 2005a).

2.7. Gas pycnometry

The apparent particle density of spray-dried powders was analyzed with nitrogen gas pycnometry (AccuPyc® 1330, Micromeritics, USA), with a 1-cm^3 sample cell. The sample cell, filled to 2/3 with powder, was purged with nitrogen ten times before performing ten analysis runs. The pressure during purges and runs were 134.4 kPa (19.5 psig) and the equilibrium rate was 34 Pa/min (0.005 psig/min).

2.8. Dissolution properties

In an effort to illustrate the dissolution behavior of *in situ* coated particles 50 mg of spray-dried sample was added to 1 ml of water (18 °C) in a 1.5 ml vial. The sealed vials were continuously rotated on a Heidolph Duomax 1030 rocking table (Rose Scientific 1030, Edmonton, Canada) and the time for dissolution, as determined by visual inspection, was recorded.

2.9. Differential scanning calorimetry (DSC)

The thermal properties of raw materials and spray-dried powders were examined with differential scanning calorimetry (DSC) (822^e, STAR^e System, Mettler Toledo, USA). Typically 5 mg of sample was carefully weighted into 40 μl pinholed Al pans. The powders were scanned twice around the expected glass transition to eliminate the effect of enthalpic relaxation. The T_g determined in the second heating scan was used for comparison between samples (Table 1). The instrument was calibrated using indium (mp 156.7 °C, $\Delta H_{melt} = 28$ J/g).

2.10. Fourier transform infrared spectrometry (FTIR)

Fourier transform infrared spectra were obtained by a Nicolet spectrometer (5DXB Thermo Electron Corp., USA) with a MCT detector in nitrogen atmosphere. For solid analysis, spray-dried powder was dispersed in 300 mg of dry KBr to obtain a protein:KBr ratio of 1:1000 and pressed into tablets under vacuum at a load of 100 MPa. For liquid analysis, spray-dried powder was reconstituted in water to a concentration of 10 mg/ml of BSA and applied to a ZnSe ATR crystal. The spectral resolution was 4 cm^{-1} and the number of scans was typically 256–512.

The absorbance spectra of protein were obtained by subtraction of absorbance spectra of placebo from the sample spectra (Dong et al., 1990). Uncompensated water vapor was subtracted from the protein (resultant) spectra (Dong et al., 1990). Second derivative spectra were obtained by a nine-point Savitsky-Golay function (OMNIC™ ESP 4.1b, Thermo Electron Corp., USA). Derivative spectra were baseline corrected and area-normalized (Kaleida Graph 3.6, Synergy Software, Reading, PA) in the amide I region (1720–1580 cm^{-1}) to allow quantitative comparisons of integrated areas under the bands (Dong et al., 1990).

2.11. Circular dichroism (CD)

Far UV spectra were obtained by a JASCO J715 spectropolarimeter (Jasco Inc., Easton, MD) at wavelengths between 180 and 275 nm. Spray-dried powders were dissolved in water to obtain a protein concentration of 5 mg/ml, which was similar to the concentration before spray-drying. Reconstituted samples were diluted with buffer to obtain a typical concentration of 0.12 mg protein/ml for use with a 1 mm path length. Each spectrum was an average of three scans.

2.12. Gel filtration

Gel filtration was conducted using a Superdex 200 26/60 column with ÄKTA Explorer 100 (Amersham Pharmacia Biotech, Uppsala, Sweden) and UV detection at 280 nm. Typically 5 ml of filtered sample (2 mg/ml of protein) was loaded on the column at a flow rate of 2.5 ml/min. The mobile phase was 10 mM sodium phosphate buffer with 0.15 M NaCl, pH 7.0. The amounts of soluble aggregates and monomer were calculated from peaks eluted after approximately 165 and 193 min. Results were reported as peak percentage area of the total peak area. The presence of insoluble aggregates in rehydrated samples was estimated by UV absorbance at 280 nm (UV/vis spectrometer Lambda 18, Perkin-Elmer, Boston, MA) before and after ultracentrifugation.

3. Results and discussion

3.1. Dynamic surface tension of HPMC

Hydroxypropyl methylcellulose (HPMC) alone is a surface-active agent, even at very low concentrations (Avranas and Iliou, 2003; Arboleya and Wilde, 2005). Complete coverage of the air/water interface with HPMC results in a surface tension of approximately 44 mN/m (Machiste and Buckton, 1996). However, the time to reach equilibrium can be long, 2–5 h, depending on the polymer bulk concentration and polydispersity (Avranas and Iliou, 2003). Dynamic surface tension recorded by the pendant drop method demonstrated that "complete adsorption" of the polymer to the air/water interface appeared already after 13 s (45–47 mN/m) even at the lowest concentrations tested here (Fig. 2a). Arboleya and Wilde (2005) obtained similar results measuring the surface tension after 10–20 min, in solution of 0.001–0.75% (w/w) of HPMC. However, for coating purposes, concentrations less than 0.001% (w/w) appeared insufficient since the surface tension remains unchanged for times as long as 1 min (induction phase) (Avranas and Iliou, 2003). Our measurements suggested that approximately 0.1% (w/w) (1% (w/w) of dry weight) of HPMC was needed for efficient coating of BSA since then the initial surface tension (<0.07 s) for HPMC

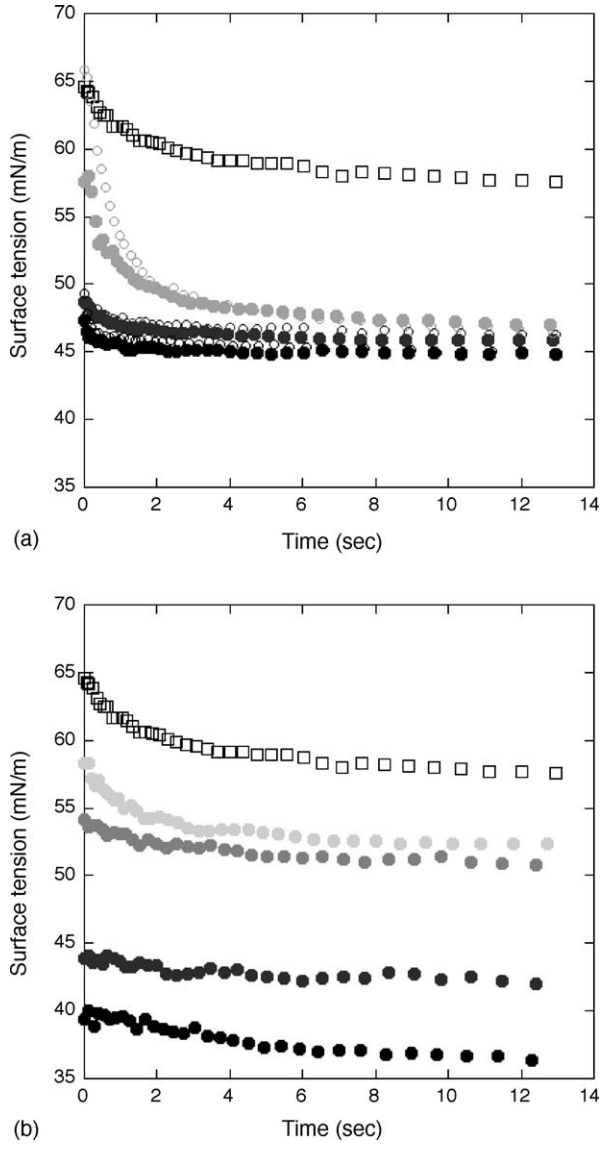

Fig. 2. Dynamic surface tension of BSA/trehalose solution after addition of increasing amounts (indicated by darker spots) of: (a) 0.01%, 0.1% and 1% (w/w) of HPMC, and (b) 0.01%, 0.1%, 1% (w/w) and 3.6% (w/w) of Poloxamer. No polymer added (squares). No BSA added (unfilled circles).

alone was considerably lower (>15 mN/m) than that of BSA and the dynamic surface tension in the mixture of BSA and HPMC was similar to that of HPMC alone (Fig. 2a). However, at a concentration of 0.01% (w/w) of HPMC the initial surface tension of the HPMC–BSA mixture was only 5 mN/m lower than that of BSA alone and it was unclear whether this would be sufficient for coating of BSA (Fig. 2a). Further, a polymer concentration higher than 1% (w/w) does not necessarily lead to a more efficient coating. An unusual behavior with increasing dynamic surface tension with increasing concentration (1–5%, w/w) of HPMC was first reported by Machiste and Buckton (1996), which attracted attention to the influence of solution viscosity on the polymer diffusion rate. Later Arboleya and Wilde (2005) found a similar behavior even at concentrations as low as 0.02 wt.%, possibly connected to the polydispersity or degree of substitution. However, this was not confirmed in our experiments.

3.2. Dynamic surface tension of poloxamer

The equilibrium surface tension of poloxamer is comparable to that of HPMC (∼40 and 44 mN/m, respectively) (Alexandridis et al., 1994; Machiste and Buckton, 1996). However, the dynamics of the polymers in relation to surface tension appeared substantially different, at the times studied here. While HPMC gradually lowered the surface tension of the fresh surface, the initial adsorption of the poloxamer appeared much faster, observed as an "instant reduction" of the surface tension (Fig. 2b). The surface tension of HPMC then converged to near-equilibrium values while the poloxamer solutions appeared comparatively slow in reaching the equilibrium surface tension. This

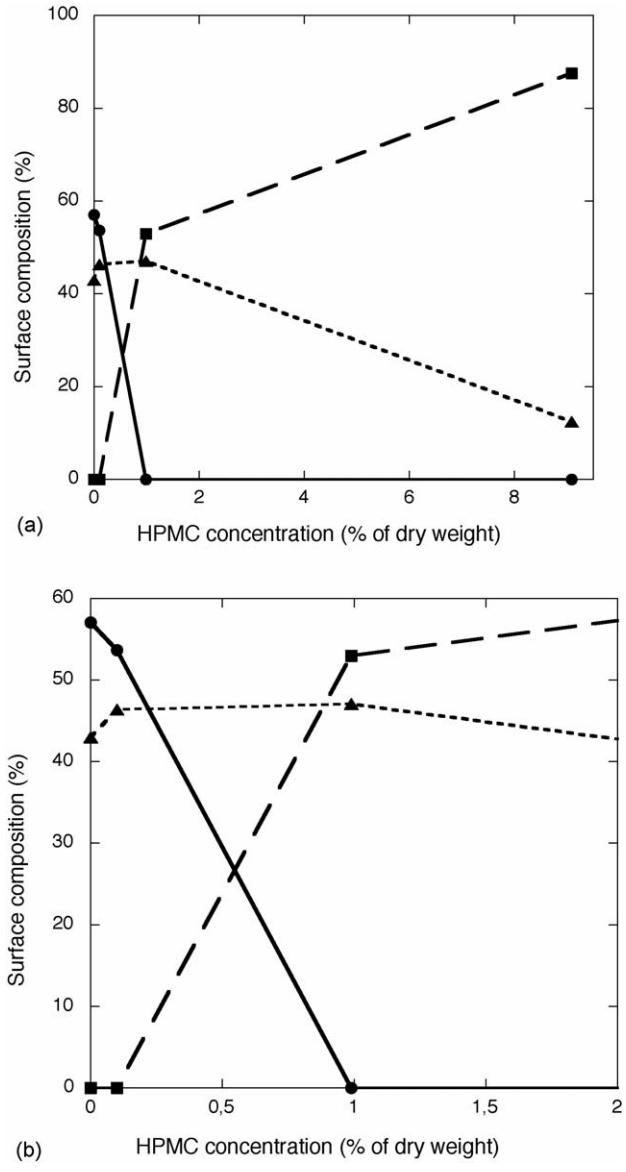

Fig. 3. Surface composition of *in situ* coated particles estimated by ESCA. Percentage coverage by BSA (●), trehalose (▲), and HPMC (■) as a function of the polymer concentration: (a) 0–9% (w/w) dry weight of polymer, and (b) 0–1% (w/w) dry weight of polymer.

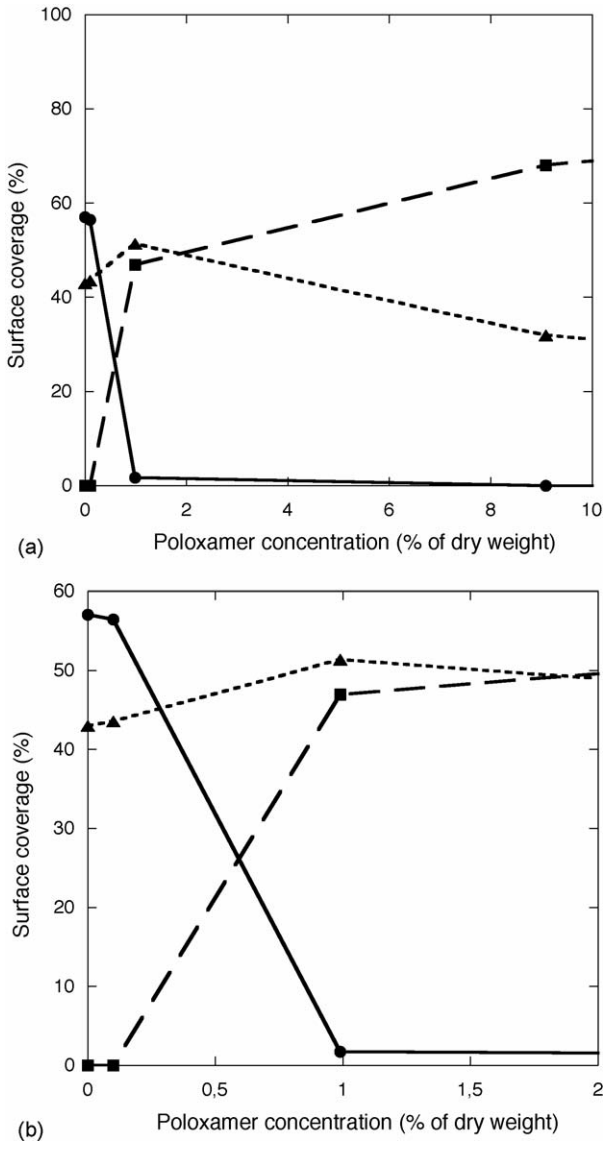

Fig. 4. Surface composition of *in situ* coated particles estimated by ESCA. Percentage coverage by BSA (■), trehalose (▲), and poloxamer (■) as a function of the polymer concentration: (a) 0–9% (w/w) dry weight of polymer, and (b) 0–1% (w/w) dry weight of polymer.

Table 2
Atomic concentration determined by ESCA for spray-dried particles coated with HPMC and Poloxamer, respectively

Sample	Atomic concentration (%)		
	C (1s)	O (1s)	N (1s)
BSA	67.1 ± 0.3	17.3 ± 0.3	15.7 ± 0.6
Trehalose, spray-dried	57.1 ± 0.0	42.9 ± 0.0	–
HPMC	64.5 ± 0.3	35.5 ± 0.3	–
Poloxamer 188	71.7 ± 0.4	28.3 ± 0.4	–
BSA/trehalos, spray-dried (5:95)	61.6 ± 02	28.5 ± 0.3	10.0 ± 0.2
BSA/trehalos/HPMC, spray-dried (9.1% polymer)	63.6 ± 0.1	36.5 ± 0.1	0.00
BSA/trehalos/poloxamer, spray-dried (9.1% polymer)	67.0 ± 0.5	33.0 ± 0.5	0.00

Mean value ± S.D. ($n = 3$).

is in accordance with literature (Blomqvist et al., 2005), and the time to reach equilibrium surface tension can be extremely long (days), due to polydispersity and slow exchange rates between the adsorbed layer and the bulk. All formulations containing both BSA and poloxamer followed the dynamics of the polymer which was expected from the reported solution diffusion coefficients of Poloxamer 188 (9.2×10^{-9} m^2/s) (Munoz et al., 2000b) and BSA (6.7×10^{-11} m^2/s) (Shen and Probstein, 1977).

3.3. Chemical surface composition of in situ coated particles

The atomic surface composition of pure materials and selected samples are shown in Table 2, and these data were used to calculate the surface coverage of the different materials on sample powders as presented in Figs. 3 and 4. The percentage surface coverage of polymer and protein correlated well with the findings of dynamic adsorption as measured by the pendant drop technique. In particles spray-dried from solutions of 1% (w/w) of dry weight of polymer no protein or only low levels (1.7% surface coverage) of protein were detected on the surface of the particles coated by HPMC and poloxamer, respectively (Figs. 3 and 4). At concentrations lower than 1% (w/w) of dry weight increasing levels of BSA were detected at the powder surface, and at the lowest amount of coating polymer used (0.1% in solids), it was not possible to calculate the surface coverage of the coating polymer. The reason for this is presumably that the C/O ratios in trehalose and polymer are not sufficiently differ-

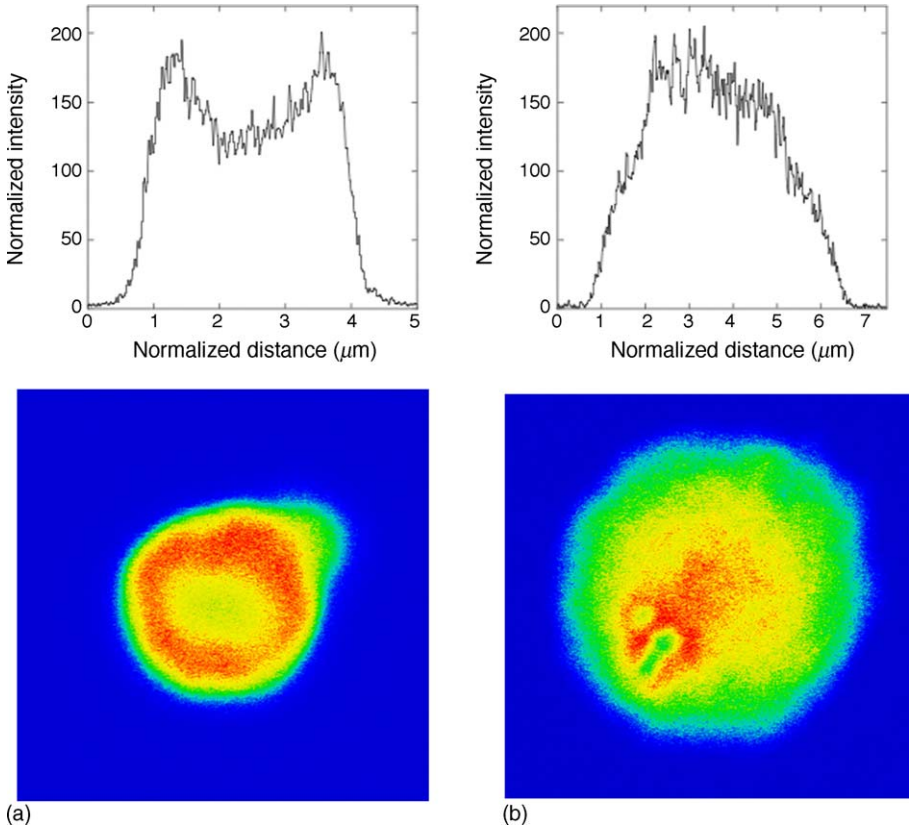

Fig. 5. CLSM cross-section illustrating the distribution of FITC-BSA in a particle before (a), and after (b) *in situ* coating. The distribution of FITC–BSA is correlated to the intensity profile showed underneath.

ent to a give a low detection limit of either of these compounds when using the 'patch' model. However, the surface coverage of BSA could be estimated with good accuracy since N is exclusive to BSA. The decreasing level of BSA at the surface at even the lowest coating polymer concentration, and the appearance of the corresponding placebo particles, suggest that the coating polymer indeed was present at the powder surface, albeit at a low level. Uncoated particles displayed a mixed surface of BSA and trehalose, containing approximately 57% of BSA (Figs. 3 and 4). This level is lower compared to what has been observed for BSA spray-dried from other carbohydrate solutions (Fäldt and Bergenståhl, 1994; Landström et al., 2000). Increasing the polymer content above 1% (w/w) resulted in higher levels of polymer at the surface. However, at a polymer concentration of 9.1% (w/w) of dry weight the surface coverage of polymer was 30% higher with HPMC compared to poloxamer (Figs. 3 and 4). Even at a concentration as high as 26% (w/w) of dry weight poloxamer the surface level was comparable to that of HPMC at 9.1% (w/w) of dry weight (data not shown). This can be presumably explained by the poloxamer forming a thinner film than HPMC, thus the ESCA signal is a combination of a (reasonably) complete surface film of poloxamer and the underlying material containing carbohydrate as well as protein. Interestingly, no signal is detected from the protein. This might indicate a steric effect from the adsorbed poloxamer layer, with the PEO-tails pointing towards the solution at higher concentrations (Munoz et al., 2000a; Blomqvist et al., 2005) Thereby, globular BSA but not trehalose might be excluded from the (sub)surface layer. The CLSM pictures in Fig. 5 illustrate suppression of BSA from the particle surface by addition of HPMC. The concentration of BSA was much higher near the particle surface in uncoated particles whereas the coated particles have a particularly high concentration of BSA towards the centre of the particle.

3.4. Surface composition and correlations to particle properties

As part of this investigation we wanted to investigate whether specific properties of the particles were affected at the polymer concentrations appropriate for *in situ* coating. Indeed, variation in the surface composition induced changes in a number of particle properties such as the particle surface morphology, particle shape, particle size, dissolution time and powder flowability.

As expected, spray-dried particles containing proteins displayed a characteristic raisin-like morphology (corrugated particles), due to the adsorption of protein at the air/liquid interface of the droplets in the spray (Fig. 6a). During drying, proteins form a visco-elastic adsorbed layer covering the surface of the droplets. Addition of a low-molecular weight surfactant, such as

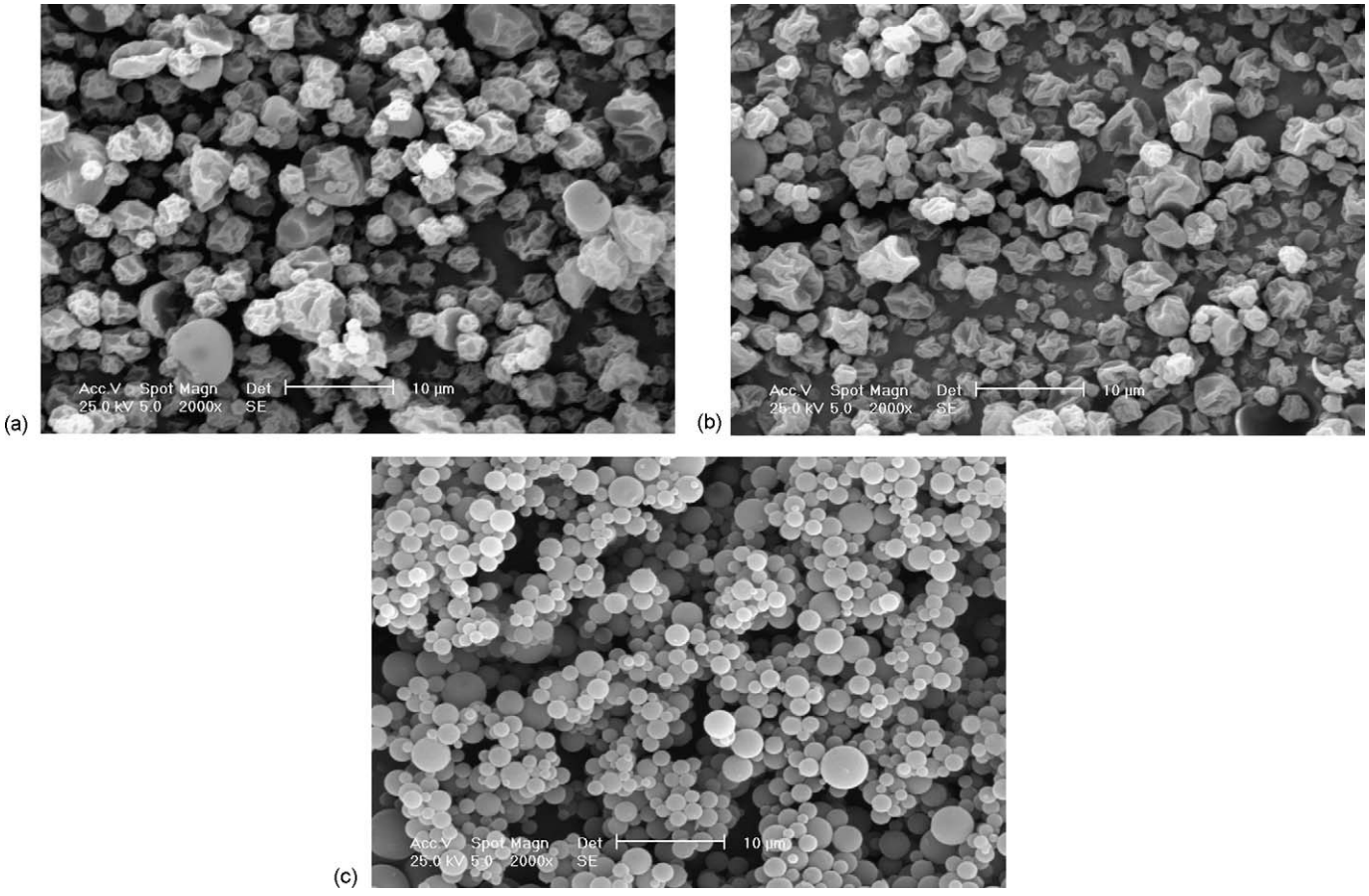

Fig. 6. Micrographic pictures of BSA-containing *in situ* coated particles: (a) uncoated, (b) HPMC, and (c) Poloxamer. The polymer content was 1% (w/w) of dry weight.

Table 3
Literature data on surface rheology of polymer and protein films

Polymer	Surface concentration (mg/m^2)	Elasticmodulus G'(mN/m)	Dilatational modulus E (mN/m)	Reference
HPMC		∼120		Arboleya and Wilde (2005)
BSA	1.37		59	Benjamins and Lucassen-Reynders (1998)
	1.95		69	
			∼60	Pereira et al. (2003)
PVA	2.2		11	Benjamins and Lucassen-Reynders (1998)
	3.1		11	
Poloxamer P85		∼0.1	2	Blomqvist et al. (2005)

polysorbate, not only expels protein molecules from the interface; it also produces a "less cohesive" film, which may result in smooth spherical particles (Maa et al., 1998; Adler et al., 2000). In terms of surface rheology, this means a film with negligible surface elasticity due to the high mobility of the surfactant in the adsorbed layer (Arboleya and Wilde, 2005). Linear film forming polymers, such as polyvinyl alcohol (PVA), have a similar effect and the change in particle surface morphology, from corrugated to smooth, is correlated to the PVA content of the particle surface (Elversson and Millqvist-Fureby, 2005a). HPMC is a strong film former and appears to form a similar type of film at the spray droplet as proteins, since the particle morphology is affected in a similar way as for protein containing particles (Fig. 6b). Possibly there is a correlation between the visco-elastic properties of the droplet surface and the morphology of spray-dried particles. To verify this hypothesis, data on surface viscosity found in literature for HPMC, BSA, PVA and poloxamer were collected and it was possible to rank these polymers according to their flexibility and surface rheology (Table 3). For example, the elastic modulus of HPMC was 130 mN/m (Arboleya and Wilde, 2005) whereas the dilatational modulus (E) of both PVA and poloxamer was comparatively lower, 11 mN/m (Benjamins and Lucassen-Reynders, 1998) and 2 mN/m (Blomqvist et al., 2005), respectively. Consequently, with a dilatational modulus of approximately 60 mN/m (Benjamins and Lucassen-Reynders, 1998; Pereira et al., 2003) this places BSA between HPMC and PVA. In addition, low-molecular surfactants, such as polysorbates are very mobile and hence, without surface visco-elasticity, which may explain the frequent observations of smooth spheres after addition of, *e.g.* polysorbates (Maa et al., 1998; Adler et al., 2000). Consequently, it might be possible to predict which polymers are likely to change the surface morphology. For example, the surface of particles without any BSA or HPMC (pure trehalose) was smooth and particles were spherical (not shown). Addition of BSA changed the morphology from smooth to highly wrinkled, as was expected from the high surface modulus of BSA (Fig. 6a). Similarly, formulations without any BSA but with an increasing content of HPMC changed in morphology from completely smooth spheres (0% HPMC) to corrugated particles (not shown). Replacing HPMC with poloxamer during *in situ* coating restored the smooth surface of spray-dried protein particles (Fig. 6c). This was in accordance with the data on surface rheology and hence, like low-molecular weight surfactants the poloxamer is able to displace proteins at the air/liquid interface and like low-molecular weight surfactant the poloxamer is mobile at the surface (Blomqvist et al., 2005).

The size of droplets and hence particles during spray-drying can be affected in opposite directions by either decreasing the surface tension or increasing the viscosity of the spray solution (Kim and Marshall, 1971; Masters, 1991). In contrast to both uncoated and HPMC coated particles all poloxamer-coated particles were less than 5 μm in diameter (Fig. 6). This effect was particularly evident at a poloxamer content of 1% (w/w) of dry weight and particles produced from solutions with both lower and higher contents of polymer were generally larger (data not shown), which may indicate an interactive effect between the initial surface tension and the liquid viscosity on the droplet size during atomization.

Studies with gas pycnometry, showed a correlation between the polymer concentration and the apparent particle density of the spray-dried powder (Table 4). However, the effect was considerably more pronounced with the HPMC coating compared to the poloxamer coating. It is likely that gas pycnometry was assessing the gas permeability of the particle surface to some extent and that the higher penetration of gas in poloxamer-coated particles result from a lower film thickness. Similar reductions in the apparent particle density with increasing concentration of polymer were observed in a previous study on

Table 4
Apparent particle density by gas pycnometry of *in situ* coated particles

Polymer (% of dry weight)	BSA/trehalose/HPMC (g/cm^3)	Trehalose/HPMC (g/cm^3)	BSA/trehalose/poloxamer (g/cm^3)
0	1.51	1.54	1.51
0.1	1.50	1.53	1.49
1	1.46	1.47	1.48
9	1.23	1.24	1.43
26	–	–	1.41

Mean value ± S.D. <0.006 ($n = 10$).

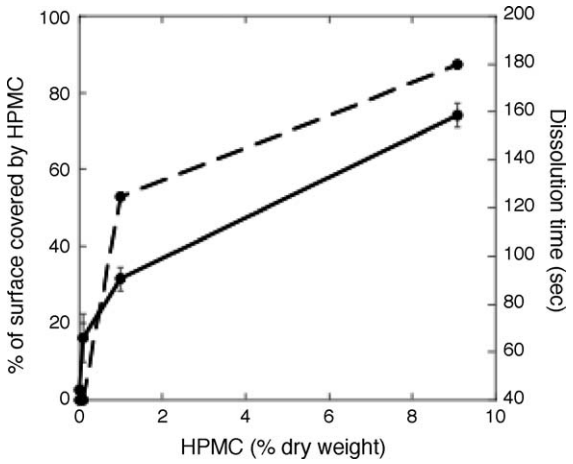

Fig. 7. Dissolution time as a function of the level of HPMC at the particle surface and the bulk concentration of HPMC.

spray-dried aqueous two-phase systems (ATPS). In formulations with an increasing content of PVA the apparent particle density decreased linearly (Elversson and Millqvist-Fureby, 2005a). The increasing surface load of PVA was confirmed by ESCA (Elversson and Millqvist-Fureby, 2005a).

A possible application for *in situ* coated particles is for modified release purposes. Although the amount of polymer is low compared to conventional coating solutions, a three-fold increase in the dissolution time was observed in the concentration span investigated (Fig. 7). The dissolution time correlated with the surface load of HPMC, as determined by ESCA. Uncoated particles dissolved similar to particles coated with the lowest concentration of HPMC. Hence, the dissolution time depended on the surface coverage rather than the concentration in the bulk. Consequently, choosing a polymer with appropriate dissolution properties *in situ* coating can be used to obtain a desired dissolution profile of the powder.

Interestingly, better flow properties of particles coated with poloxamer compared to HPMC were noticed during spray-drying and powder handling. Possibly, interparticulate interactions were affected by either surface composition or particle shape and morphology (Hickey et al., 1994). For example, the water sorption isotherm of poloxamer is below that of HPMC (Kibbe, 2000), resulting in a less "sticky" powder. However, it was not possible to confirm these results by flowability tests and close inspections of SEM images implied that the primary particles in samples with a high poloxamer content were agglomerated into small aggregates, which may in part account for the improved flow properties.

Inclusion of polymeric material can influence the recrystallization behavior of, *e.g.* spray-dried lactose (Stubberud and Forbes, 1998; Corrigan et al., 2002; Berggren and Alderborn, 2004). However, it was unclear whether very low concentrations of polymer as in *in situ* coating would affect the solid-state properties of the particles. The spray-dried particles consisted mainly of trehalose. Consequently, it was the transitions of trehalose that appeared from DSC analysis. Addition 5% of BSA induced a moderate (3 °C) increase of the glass transition temperature of the dried powder from 118.5 to 121.5 °C (Table 5). However, addition of HPMC, which has a T_g around 175 °C, did not, at the studied concentrations, affect the T_g of the formulations that remained amorphous. Neither was any effect observed on T_g from addition of poloxamer. The T_g of poloxamer can be assumed to be similar to that of PEG (Kibbe, 2000), *i.e.* lower than all other constituents, and particles coated with poloxamer showed the presence of crystalline polymer, with a melting transition at approximately 50–53 °C (Table 5). Apparently, the addition of an amphiphilic polymer, such as HPMC or poloxamer result in phase separation close to the surface during spray-drying, due to the surface activity of the polymer. In contrast, addition of, *e.g.* dextran, which has no surface activity, forms amorphous particles with a T_g dependant of the mass content of each excipient (Elversson and Millqvist-Fureby, 2005a).

3.5. Structural integrity of protein of in situ coated particles—FTIR, CD and gel filtration

Bovine serum albumin (BSA) in all *in situ* coated particles had a native-like structure comparable to that of rehydrated BSA, as observed from CD, FTIR and gel filtration. BSA supplied from the commercial source and dissolved in buffer, had a α-helix content of approximately 55%, which was expected (Peters, 1995). Further, the content of 89% monomer and 11% of soluble aggregates were values typical of a commercial sample. In comparison, only slightly lower levels of α-helix (47%) were detected by FTIR in the liquid-state, for samples coated with HPMC (Table 6). In the dried state, the high preservation of native structure in *in situ* coated samples as displayed by FTIR was confirmed by oförändrade levels of soluble aggregates (Table 6). Poloxamer-coated samples were not analyzed with FTIR but both CD (Fig. 8) and gel filtration (Fig. 9) confirmed that a native structure was most likely. The structural integrity

Table 5
DSC results on thermal transitions in spray-dried powders coated with HPMC and poloxamer, respectively

Polymer (% of dry weight)	BSA/trehalose/HPMC	Trehalose/HPMC	BSA/trehalose/poloxamer		
	T_g (°C)	T_g (°C)	T_g (°C)	T_m (°C)	ΔH_m (J/g)
0	121.6 ± 0.1	118.5 ± 0.5	121.6 ± 0.1	–	
0.1	121.1 ± 0.3	122.1	121.6	–	–
1	121.4 ± 0.5	122.2 ± 0.1	120.5 ± 0	50.5 ± 0.1	0.2 ± 0.1
9	121.6 ± 0.2	123.0 ± 1.5	121.4	51.1 ± 0	4.1 ± 0.1
26	–	–	121.4 ± 0.2	52.6 ± 0.1	24.8 ± 0.5

HPMC: T_g,~170–180 °C (Kibbe, 2000); Poloxamer: T_m, 55 °C, ΔT_m, 115J/g, T_c, 30 °C. Mean value ± S.D. (n = 3).

Table 6
Structural integrity of protein in *in situ* coated particles as analyzed with CD, FTIR and gel filtration

Excipients	Polymer (% of dry weight)	Protein:trehalose mss ratio	CD[a]	FTIR[b]	SEC[c]		
				α	*N*	Aggr	Frag
BSA, native	–	–	N	55	89	11	–
Trehalose, dextran	–	1:3.8	–	33	34	2 (13)	51
Trehalose	0	1:19	N	43	88	12	–
In situ HPMC, trehalose	0.1	1:19	N	49	89	11	–
	1	1:19	N	45	89	11	–
	9	1:19	N	47	89	11	–
In situ poloxamer, trehalose	0.1	1:19	na	na	86	14	–
	1	1:19	N	na	89	11	–
	9	1:19	N	na	na	na	na
	26	1:19	N	na	na	na	na

na, not analyzed; N, native BSA; α-helix content (%); Aggr, soluble aggregates (insoluble); Frag, fragments (%).

of BSA in uncoated particles was higher than anticipated, and comparable to that of BSA in coated particles. BSA spray-dried with trehalose alone had a slightly lower α-helix content (43%), and approximately 12% of soluble aggregates, compared to 11% in native or *in situ* coated samples (Table 6). In comparison, both α-helix and monomer content was substantially lower (33% and 34%, respectively) as BSA was spray-dried in a formulation with trehalose and dextran in ratio 1:5 (Table 6 and Fig. 9). Possibly, the stabilizing effect of trehalose dominated over the coating effect, since at a protein concentration of 5% (w/w) in the powder only approximately 4% of the total content of protein can be expected at the particle surface (Landström et al., 1999). A lower protein concentration in the spray solution would have resulted in a higher adsorbed fraction, and possibly a significant as well as concentration dependent effect of the *in situ* coating. However, due to the limitations regarding the protein concentration needed for liquid FTIR, the protein concentration of the spray solutions was set to 5 mg/ml.

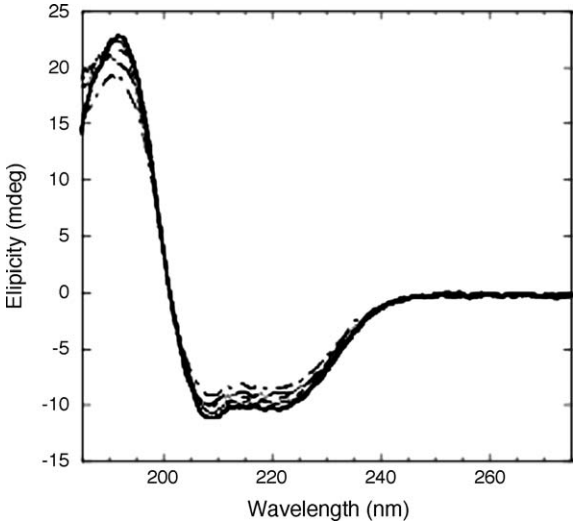

Fig. 8. CD spectra of *in situ* coated particles after rehydration. Native BSA (bold line), uncoated (—), 0.1% (w/w) (— – —), 1% (w/w) (– – –), 26% (w/w) (- - -) of dry weight poloxamer.

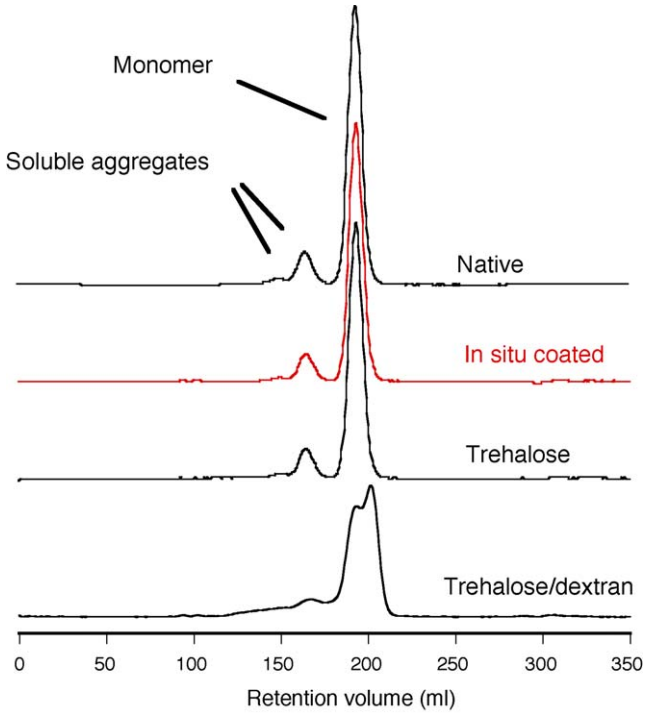

Fig. 9. Content of monomeric and aggregated BSA by gel filtration.

4. Concluding remarks

Dynamic surface tension at the air/liquid interface, as measured by the pendant drop technique was very useful in prediction of the chemical composition of the spray-dried particle surface. Due to the very short lifetime of droplet surfaces during spray-drying adsorption kinetics of surface-active components become more important than the equilibrium surface tension and hence, molecules adsorbed at the surface in the moment of shell formation will remain there during drying. Consequently, the correlation between dynamic surface tension and ESCA was very satisfying (Figs. 2–4). Efficient coating, with full coverage of BSA appeared at a polymer concentration ≥1% (w/w) of dry weight and the repression of BSA from the surface was

illustrated by CLSM of FITC-labeled BSA (Fig. 5). Further, changes in the surface morphology of particles (Fig. 6) might be correlated to differences in surface visco-elasticity between polymers. In addition, the *in situ* coating induced a change in several particles properties of pharmaceutical relevance, such as time to dissolution and flowability. Moreover, studies with gas pycnometry, showed a correlation between the polymer concentration and the apparent particle density of the spray-dried powder (Table 5), presumably due to changes in the gas permeability of the surface. The effect was more pronounced for HPMC coated particles than for poloxamer-coated particles and might indicate an effect of the thickness of the polymer film. Although it was not possible to detect any improved conformational stability of protein from *in situ* coating, the results do not exclude that additional stabilization would be achieved in a low-dosage formulation where the fraction of surface adsorbed protein is expected to be higher (Table 6).

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