Surface Composition of Spray-Dried Particles of Bovine Serum Albumin/ Trehalose/Surfactant

Michael Adler,[1] Michael Unger,[2] and Geoffrey Lee[1,3]

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Purpose. To characterize via electron spectroscopy for chemical analysis (ESCA) the surface of spray-dried particles of trehalose plus a protein (bovine serum albumin). Additionally, to show how and why the addition of a surfactant reduces protein adsorption, and by this mechanism could reduce protein instability during spray-drying.

Methods. Aqueous solutions of trehalose plus bovine serum albumin (bSA) were spray-dried with increasing concentrations of surfactant. The surface composition of the dried particles was examined using ESCA.

Results. The presence of bSA, trehalose, and surfactant could be detected quantitatively in the particle surface. In the absence of surfactant the bSA had a large surface excess concentration (determined via its N atoms). Increasing concentration of polysorbate 80 reduced the surface excess of bSA in a concentration-dependent manner. At high polysorbate 80 concentration (5 mg/ml) the bSA could no longer be detected in solid surface. Using sodium dodecyl sulfate it was shown that the reduction in surface concentration of the protein is accompanied by a simultaneous increase in surface concentration of the surfactant. Neither surfactant fully covers the surface at the point of complete protein exclusion.

Conclusions. ESCA provides a direct, quantitative measure of the surface composition of spray-dried trehalose/protein/surfactant particles. Surfactant reduces protein adsorption at the water/air-interface. This appears to be a result of complex formation with the surfactant within the bulk spray solution.

KEY WORDS: protein; spray-dry; surfactant; surface-composition; ESCA.

INTRODUCTION

Solutions of peptides or proteins can be readily spray-dried to produce fine particles in the size range suitable for pulmonary delivery. Stabilizing adjuvents added to the spray solution are always necessary to improve process- and storage-stability (1,2). These are typically, but not exclusively, disaccharides (3) that form amorphous glasses on spray drying (4). The reason for their stabilizing action during the spray-drying process is most likely water replacement (5). Their stabilizing action during storage of the dried product may be glassy immobilization (4), although several hypothyses exit. Hemoglobin (2), β-galactosidase (3), lactate dehydrogenase (LDH) (4) and trypsinogen (5) could be successfully spray-dried in this way.

A number of factors have been considered to produce peptide/protein damage during spray-drying: shearing stresses in the nozzle (6), thermal stress during droplet drying in the spray-drying tower (3, 4), and peptide/protein-adsorption at the greatly expanded liquid/air interface of the spray solution on atomization (1). Recent studies by Maa *et al.* (1,7) have shown, however, that aggregation of carbohydrate-free recombinant human growth hormone (rhGH) during spray-drying at an air inlet temperature, T_{inlet}, of 90°C was induced primarily by adsorption at the liquid/air interface created during spraying; shearing and thermal stresses were considered to be of minor importance for this protein. For this reason surfactants can act as stabilizing adjuvents during spray-drying. The addition of polysorbate 20 to the spray solution reduced aggregation of the carbohydrate-free-rhGH from approx. 15% to $<$2% (1). Viewed intuitively, the liquid/air interface of the spray droplets before drying would be preferentially occupied by the surfactant molecules rather than the protein (competitive adsorption), hereby reducing the amount of protein adsorbed and hence unfolded and aggregated. We recently presented quantitative evidence to support this intuitive model of protein/surfactant adsorption at the liquid/air and solid/air interfaces (4). The presence of a large excess concentration of LDH in the surface of spray-dried trehalose particles could be detected by determining elemental nitrogen using electron spectroscopy for chemical analysis (ESCA). On addition of 0.1% w/w polysorbate 80 to the spray solution, no more elemental nitrogen was detected in the surface of the spray-dried particles, indicating exclusion of LDH by the surfactant.

In this paper we present a more extensive study of protein exclusion from the solid/air-interface of spray-dried particles using ESCA. The major goal is to demonstrate that a surfactant excludes a protein from a solid/air-interface in a concentration-dependent manner. To that end we determine the relative concentrations of all 3 components, viz. protein, sugar and surfactant, in the surface of the spray-dried particles. We chose bovine serum albumin (bSA) as a model protein, and three surface-active materials: polysorbate 80, for which there is already unequivocal evidence of its stabilizing action on proteins during spray-drying (1,4); sodium dodecyl sulfate, whose presence in the surface of spray-dried particles should be directly determinable from its S-atom; and the phospolipid Lipoid E80, serving as a non-adsorbable negative control. We use ESCA to examine the presence of bSA in the surface of spray-dried trehalose particles containing surfactant in increasing concentration. Scanning electron microscopy shows that changes in the trehalose-particles' surface morphology caused by the presence of protein and surfactant correlate well with the ESCA-results. With these experiments we gain a better understanding of the heterogeneous distribution of sugar, protein and surfactant in spray-dried particles. It is suggested that improved protein stability during the process of spray-drying is more likely a result of complex formation between protein and surfactant in the bulk spray solution, than of simple competitive adsorption.

MATERIALS AND METHODS

Materials

Bovine serum albumin (bSA) of molecular weight approx. 66 kDa was obtained from Boehringer Mannheim and used

[1] Department of Pharmaceutical Technology, Friedrich-Alexander-University, Erlangen, Germany.
[2] Department of Materials Science, Friedrich-Alexander-University, Erlangen, Germany.
[3] To whom correspondence should be addressed. (e-mail: lee@pharmtech.uni-erlangen.de)

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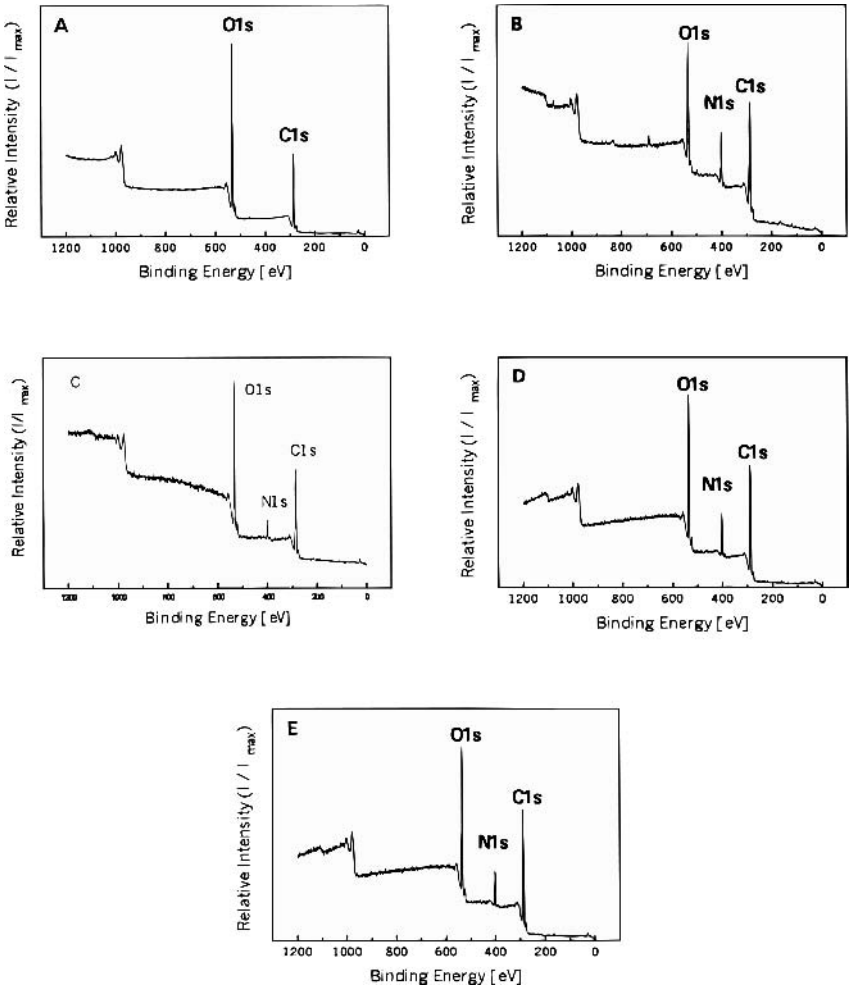

Fig. 1. ESCA spectra obtained at room temperature. (A) Spray-dried trehalose; (B) bovine serum albumin (bSA) (used as received); (C) spray-dried trehalose obtained from spray solution containing 5 mg/ml bSA and 95 mg/ml trehalose; (D) spray-dried trehalose obtained from spray solution containing 50 mg bSA and 50 mg/ml trehalose; (E) spray-dried trehalose obtained from spray solution containing 50 mg bSA, 50 mg/ml trehalose and 1 mg/ml polysorbate 80. For spray-drying conditions, see text.

Table 1. % Atomic Concentrations Determined by ESCA for Spray-Dried Trehalose, Untreated bSA, Untreated Sodium Dodecyl Sulfate, and Spray-Dried bSA/Trehalose (5 mg:95 mg).

	Atomic concentration				
Substance	C1s	N1s	O1s	S2p	Na1s
spray-dried trehalose	52.9 ± 1.4	—	46.7 ± 1.3	—	—
bovine serum albumin (bSA)	62.6 ± 1.6	14.4 ± 1.3	23 ± 2.5	—	—
sodium dodecyl sulfate	65.5 ± 1.7	—	22.9 ± 0.5	4.9 ± 0.1	6.6. ± 0.8
spray-dried bSA/trehalose	56.6	5.9	37.6		

Note: Mean value ± standard deviation (n = 4).

to prepare the spray-dried particles. Trehalose dihydrate were obtained from Sigma Chemicals (Munich, Germany). Polysorbate 80 of molecular weight approx. 1300 and HLB-value 15 was also obtained from Sigma (Munich, Germany). Sodium dodecyl sulfate was obtained from Roth Chemicals (Berlin, Germany), and Lipoid E80 from Lipoid (Ludwigshafen, Germany). Water was double-distilled from an all-glass apparatus.

Spray-Drying of Trehalose/bSA/Surfactant

Trehalose dihydrate, bSA and the appropriate surfactant were dissolved in water, and the resulting solution spray-dried on a Büchi model 190 laboratory spray dryer operated in the co-current mode. The liquid feed-rate was 4 ml/min through a pneumatic nozzle (0.7 mm diameter) driven at 6 bar air pressure. Cooled water was circulated through a jacket around the nozzle. The atomising air flow-rate was 0.7 m^3/h, at an aspirator vacuum of 38 mbar. Spray-drying was performed at an inlet-air temperature (T_{inlet}) of 150°C, corresponding to an outlet-air temperature (T_{outlet}) of 95°C. The spray solution contained 10% w/w total dissolved solids. Under these conditions pure spray-dried trehalose particles are fully amorphous and have a mean diameter by laser diffraction of 3.4 μm, a residual moisture content of 2.5% w/w, and a glass transition temperature of 78°C (4).

Electron Spectroscopy for Chemical Analysis (ESCA)

With this method we determined the composition of the surface of the spray-dried particles. ESCA determines the elements present in the near-surface region (approx. 10 nm) of the solid (8). In brief, a powder sample is exposed to an X-ray beam, and the electrons contained in the near-surface region that have a binding energy less than the photon energy will be ejected from their atoms. The kinetic energy of the ejected atoms will be equal to the difference between photon energy and their binding energy, after correction for an instrument response function. The elements present in the specimen can hereby be identified quantitatively (9), since the binding energy is characteristic of the atom from which the electron was ejected.

We wished to examine the surface composition of the spray-dried trehalose particles containing bSA and surfactant. This was achieved by analysing the surface relative % atomic concentrations of the elements C, O, N and S. A photoelectron spectrometer (Physical Electronics) was used with an AlK_α X-ray source. The pressure in the sample chamber was reduced to 10^{-9}–10^{-10} mbar. The electron kinetic-energy analyser was operated with the pass energy of 188 eV, and the ESCA spectra were determined using a step size of 1.0 eV. The result of each ESCA analysis is expressed as a spectrum of relative counts/s at the electron kinetic-energy analyser versus binding energy of the atoms in eV.

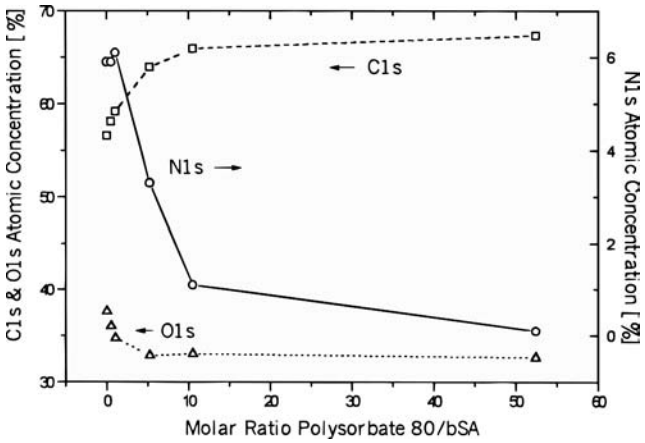

Fig. 2. Influence of molar ratio polysorbate 80/bovine serum albumin (bSA) ($\mathbf{m_{s/p}}$) in spray solution on % relative atomic concentrations of N, C, and O in spray-dried trehalose/bSA. Spray solution contained 5 mg/ml bSA and 95 mg/ml trehalose.

Scanning Electron Microscopy

The surface morphology of the spray-dried particles was examined on an Amray 1810 T microscope (SEM). The powders were gold sputtered on an Al sample holder before being examined.

RESULTS AND DISCUSSION

The ESCA spectrum of spray-dried trehalose in Fig. 1a shows 2 peaks from the 1 s electrons of O and C (H is not measured). The corresponding relative % atomic concentrations of O and C are 46.7 ± 1.3 and 52.9 ± 1.4%, respectively (Table 1), compared with the theoretical values of 48.0 and 52.0 for O_{11}/C_{12} (10). The ESCA spectrum in Fig. 1b of pure bSA (used as received) shows 1 s-electron peaks from O, C and also N. The relative % atomic concentrations of O, C and N are 23 ± 2.5, 62.6 ± 1.6 and 14.4 ± 1.3%, respectively (Table 1), also in close agreement with the theoretical values of 21.0, 64.0 and 15 (11). In Fig. 1c ESCA also clearly reveals the presence of N and hence of bSA in the surface of the particles prepared by spray-drying a solution containing 95 mg/ml trehalose and 5 mg/ml bSA. As already found for spray-dried LDH/trehalose (4), the relative % atomic concentration of N (5.9%) is approx. 9 times higher than that expected from a homogeneous distribution of bSA within the trehalose particles (approx. 0.7%). We may assume that the ratio of elements found in any spray-dried trehalose/bSA mixture is a linear combination of the ratio of the elements present in the two pure components (8). In this case the measured relative % atomic concentrations of C and O in the spray-dried trehalose/bSA particles (Table 1) correspond to a surface layer composed of approx. 40% bSA and 60% trehalose. The spray-drying solution contained, however, 5 parts bSA to 95 parts trehalose by weight. We attribute this 9-fold increase in N-signal to adsorption of bSA to the water/air interface of the atomized spray droplets before they dry. At T_{inlet} = 150°C droplet drying will be very rapid; the critical point (t_{crit}), at which solid starts to precipitate and the surface becomes immobilized (12), occurs after approx. 0.4 ms under the spray-drying conditions used here (4). If subsequent diffusional and convectional movement at the solid surface is prohibited, then the ESCA-results reflect the protein's distribution at t_{crit}. A second factor may, however, also be partly responsible for the high surface N-signal. It is likely that bSA partially unfolds on adsorption at an air/water-interface (13). Resulting exposure of the polypeptide backbone in the surface of the solid particles could increase the measured N-signal compared with correctly folded bSA. This is currently being examined for spray-dried particles using fluorimetry and FT-IR.

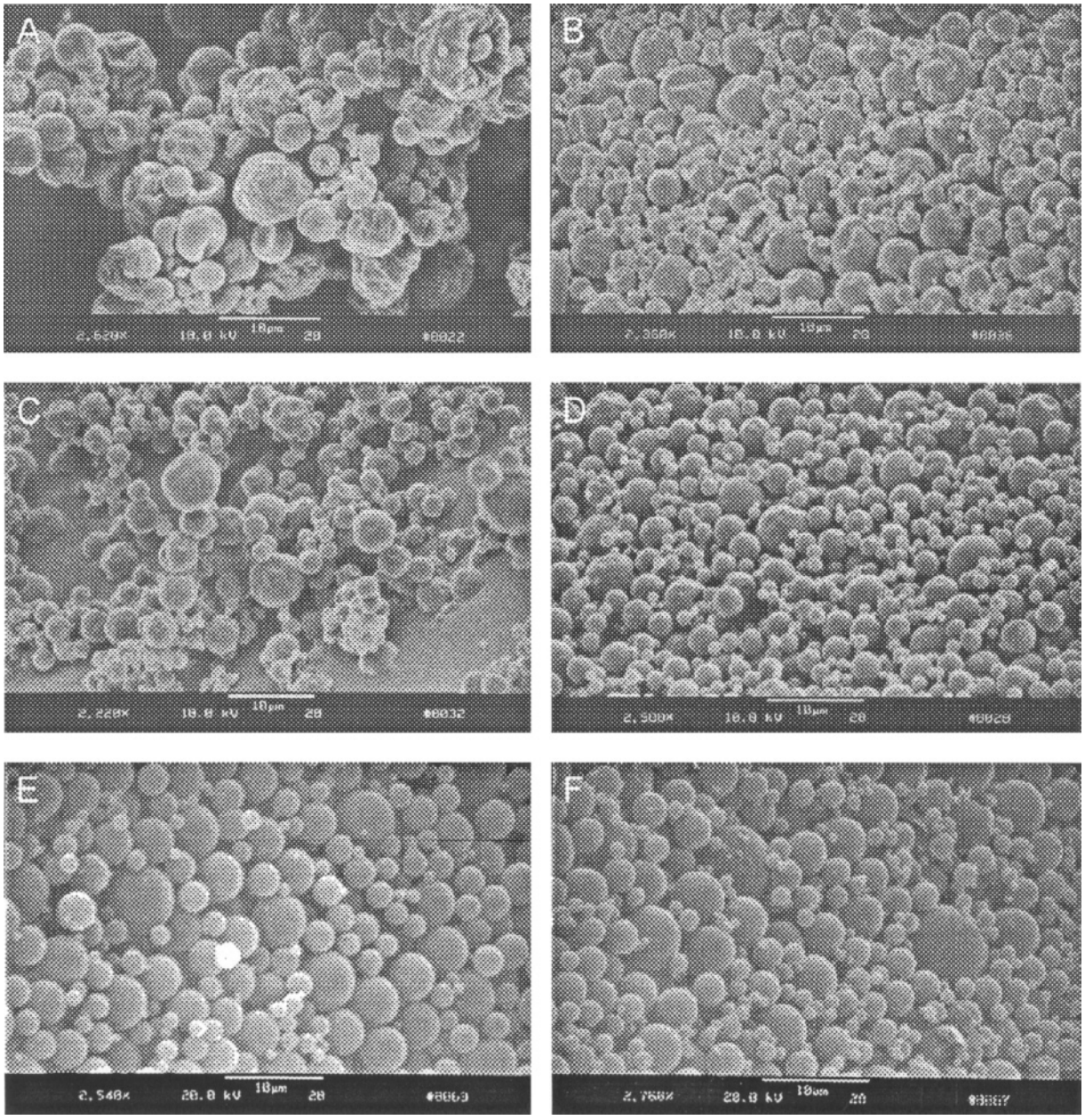

Fig. 3. Scanning electron micrographs of spray-dried bovine serum albumin (bSA)/trehalose/polysorbate 80. Spray solutions contained 5 mg/ml bSA and 95 mg/ml trehalose. (A) $\mathbf{m}_{s/p} = 0$; (B) $\mathbf{m}_{s/p} = 0.524$; (C) $\mathbf{m}_{s/p} = 1.05$; (D) $\mathbf{m}_{s/p} = 5.24$; (E) $\mathbf{m}_{s/p} = 10.5$; (F) $\mathbf{m}_{s/p} = 52.4$.

The addition of polysorbate 80 to the spray solution produces changes in all 3 elemental 1 s-electron peaks. The presence of a molar ratio of surfactant/protein, $\mathbf{m}_{s/p}$, of up to 1.0 in the spray solution gives no change in surface coverage of the spray-dried particles with N (Fig. 2). At this surfactant concentration ($\equiv$ 0.1 mg/ml in the spray solution containing 5 mg/ml bSA) there is evidently no change in the presence of bSA at the water/air interface of the atomized spray droplets up to t_{crit}. The relative % atomic concentration of C increases, however, whilst that of O decreases up to $\mathbf{m}_{s/p} = 1.0$ (Fig. 2). This is unlikely to be a result of peak interference, and indicates a changed composition of the surface layer. We did not measure the ESCA spectrum of pure polysorbate 80, since this is a liquid at room temperature and cannot be held at the high vacuum necessary in the sample chamber of the photoelectron spectrometer. We can, however, consider the result shown in Fig. 2 in terms of the known theoretical % relative atomic concentrations of C and O in the polysorbate 80 molecule, these being 70.5% and 29.5% respectively for C_{62}/O_{26} (10). If the protein concentration in the surface region remains unchanged up to $\mathbf{m}_{s/p} = 1.0$ (see N1s result in Fig. 2), then the increase in C1s and decrease in O1s in this range corresponds to displacement of the trehalose (52.9% C and 46.7% O, see Table 1) by the polysorbate 80 at the water/air interface of the drying droplets up to t_{crit}. This suggestion is supported by the known negative excess surface concentrations (Γ) of disaccharides at the water/air interface (14).

As $\mathbf{m}_{s/p}$ increases above unity, the % relative concentration

of N in the solid surface decreases steadily up to $\mathbf{m}_{s/p} = 10$, and thereafter more slowly up to $\mathbf{m}_{s/p} = 50$ (Fig. 2). We attribute this disappearance of N to progressive displacement of the bSA from the solid particle surface, depending on the amount of polysorbate 80 present. There is no apparent relation between this surface displacement of bSA and the CMC of polysorbate 80 in bulk aqueous solution. This lies at 0.01 mg/ml ($\equiv \mathbf{m}_{s/p}$ of 0.1 with 5 mg/ml bSA in spray solution) (15) at which concentration the % relative atomic concentration of N is as yet unchanged compared with $\mathbf{m}_{s/p} = 0$ (Fig. 2). It is, however, for two reasons not possible to define precisely the CMC of the surfactant within the spray solution. First, the concentration of polysorbate 80 within the bulk solution of the spray droplets will be lower than that in the original spray solution, since it will be depleted by surfactant adsorption at the expanding water/air-interface. Secondly, the surfactant's CMC will be shifted to a higher value in the presence of the dissolved second solute, i.e., bSA. At $\mathbf{m}_{s/p} >$ unity the surface concentration of C continues to increase and that of O continues to decrease (Fig. 2). This must now in part be a result of displacement of bSA from the surface. Like polysorbate 80, bSA also has a higher content of C (62.6%) and a lower content of O (23%) than the trehalose (52.9% C, 46.7% O) (cf. Table 1). At $\mathbf{m}_{s/p} \geq 10$ (Fig. 2), the changes in surface composition becomes much less marked. Taken together, the changes in the % relative atomic concentrations of C, N and O given in Fig. 2 indicate progressive exclusion of first trehalose, and then also the protein from the surface region of the spray-dried particles on increase in surfactant concentration. Although there are no stability data available for the effect of polysorbate 80 on spray-dried bSA in trehalose, these results support the argument (1,4,7) that surfactant reduces protein inactivation or aggregation during spray-drying by hindrance of protein adsorption to the water/air-interface. The composition of the solid surface at high $\mathbf{m}_{s/p} = 52.4$ (i.e., where N1s is effectively zero) is 33% O1s and 67% C1s (see Fig. 2), which does not correspond to the relative % atomic composition of pure polysorbate 80 (29.5% O and 70.5% C). A simple calculation shows that this composition of the surface layer corresponds to approx. 81% polysorbate 80 and 19% trehalose. The surface is therefore not fully covered with surfactant, the remainder being occupied by the trehalose rather than the bSA. This is surprising, since disaccharides (presumably also trehalose) have negative Γ at the water/air-interface (14), whereas bSA has a positive Γ (13). We shall return to this point again presently.

The scanning electron micrographs provide good collaborating evidence of bSA displacement by polysorbate 80 in the same range of $\mathbf{m}_{s/p}$. Trehalose particles spray-dried at $T_{inlet} = 150°C$ in the Büchi under the same conditions used here are spherical with smooth surfaces (4). As previously found for rhGH (16) granulocyte colony stimulating factor (17) and LDH (4), the presence of the protein (in this case 5 parts bSA to 95 parts trehalose) in the spray solution results in the wrinkled appearance shown in Fig. 3a. This marked change in surface morphology is typical for the effects of a high molecular weight additive, which alters the balance of surface-to-viscous forces controlling droplet shape during drying (18). Addition of polysorbate 80 to the spray solution causes a concentration-dependent return to the spherical, smooth shape seen without bSA. Up to $\mathbf{m}_{s/p} = 1.0$ ($\equiv$ 0.1 mg/ml polysorbate 80 in the spray solution) (Fig. 3b–c) there is little change in particle appearance.

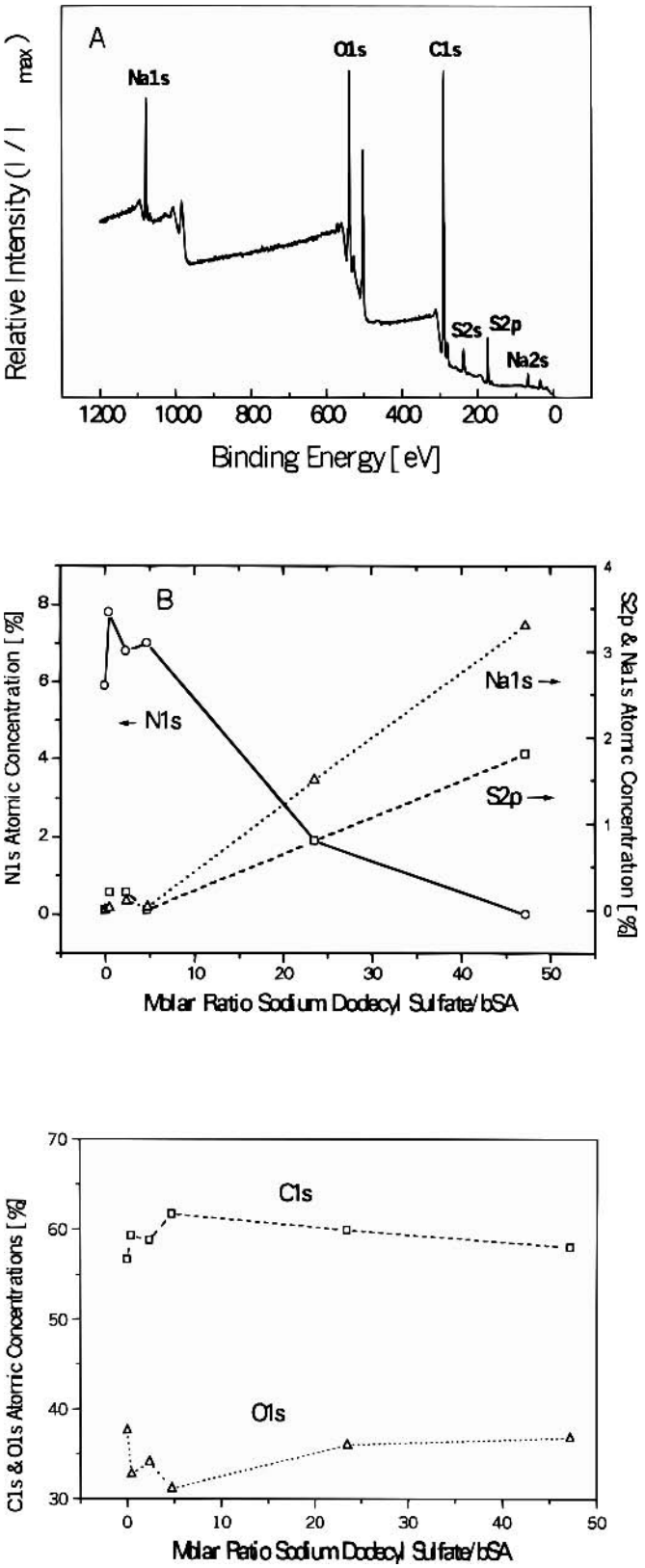

Fig. 4. (A) ESCA spectrum of pure sodium dodecyl sulfate (used as received); (B) Influence of molar ratio sodium dodecyl sulfate/bovine serum albumin (bSA) ($\mathbf{m}_{s/p}$) on % relative atomic surface concentrations of N, S, and Na in spray-dried trehalose/bSA; (C) Influence of molar ratio sodium dodecyl sulfate/bovine serum albumin (bSA) ($\mathbf{m}_{s/p}$) on % relative atomic surface concentrations of C and O in spray-dried trehalose/bSA. Spray solutions contained 5 mg/ml bSA and 95 mg/ml trehalose.

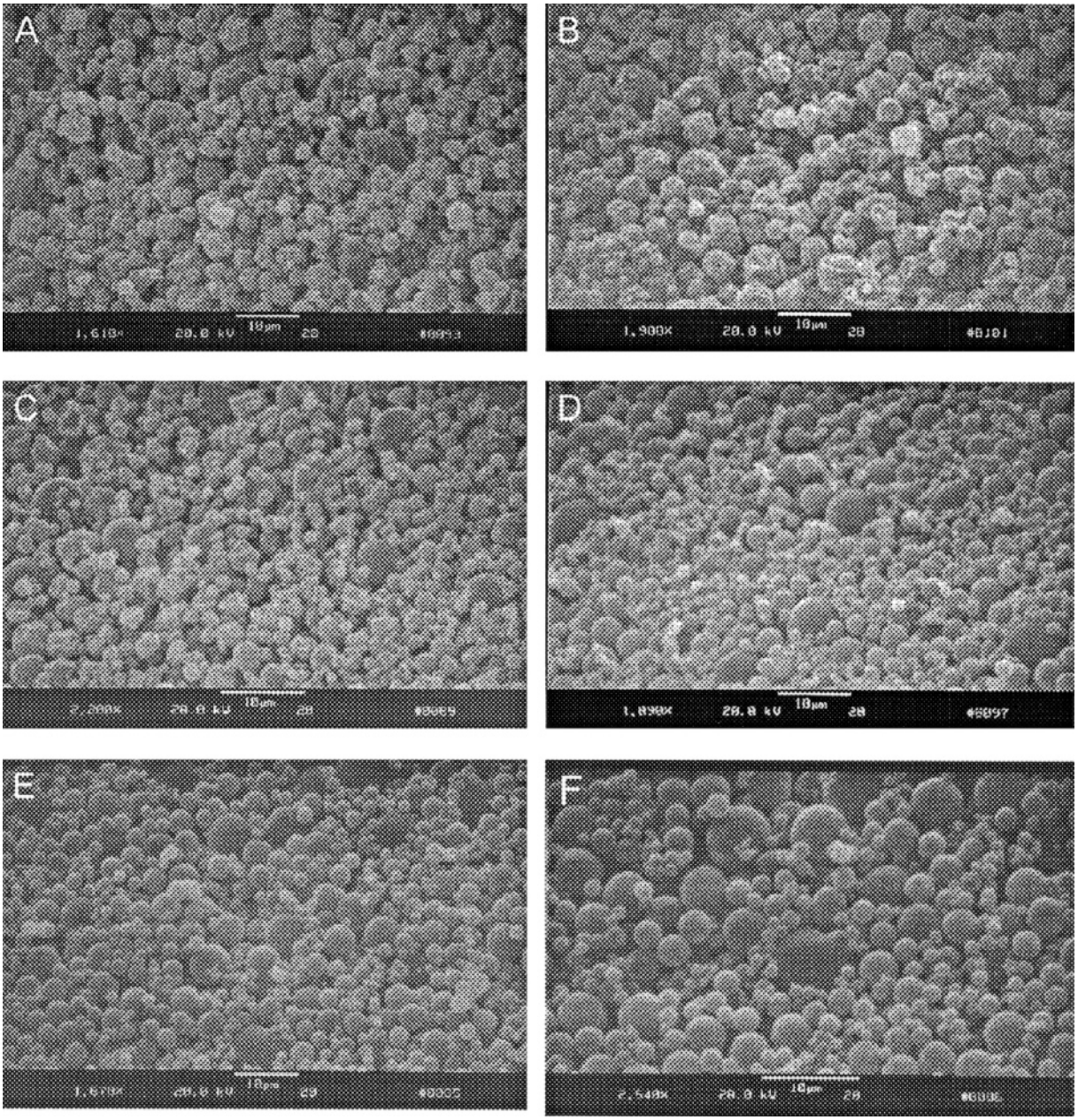

Fig. 5. Scanning electron micrographs of spray-dried trehalose/bovine serum albumin (bSA). (A) $\mathbf{m}_{s/p}$ = 0.47; (B) $\mathbf{m}_{s/p}$ = 2.35; (C) $\mathbf{m}_{s/p}$ = 4.72; (D) $\mathbf{m}_{s/p}$ = 23.5; (E) $\mathbf{m}_{s/p}$ = 47.2. Spray solutions contained 5 mg/ml bSA and 95 mg/ml trehalose. Added surfactant was Na dodecyl sulfate.

Between $\mathbf{m}_{s/p}$ = 1.0 and $\mathbf{m}_{s/p}$ = 10.5 (Fig. 3d–e), however, the particles gradually return to their spherical, smooth shape on increasing amount of surfactant. Increasing $\mathbf{m}_{s/p}$ further to 52.4 (Fig. 3f) has no further effect on the particles' appearance. Thus in that range of $\mathbf{m}_{s/p}$ were the bSA is progressively displaced from the solid particle surface ($\mathbf{m}_{s/p}$ = 1.0 − 10.5 in Fig. 2) we find a progressive improvement in surface smoothness. This is further evidence of protein displacement from the water/air interface of the spray droplets (16) up to t_{crit}, and can be explained by the reduction in surface tension of the interface by the adsorbed surfactant. This alters again the balance of surface-to-viscous forces so as to promote a smooth particle surface after drying (18). Figures 2 and 3 thus show a clear relation between surface composition and surface morphology of the spray-dried trehalose/bSA/polysorbate 80 particles.

A 10-fold increase in the bSA concentration in the spray solution up to 50 mg/ml (plus 50 mg/ml trehalose) causes a doubling of the % relative atomic concentration of N in the surface layer of the spray-dried particles up to 10.8% (Fig. 1d). The presence of 1 mg/ml polysorbate 80 in the spray solution is now only equivalent to $\mathbf{m}_{s/p}$ = 1.0, and therefore only marginally reduces the % relative atomic concentration of N to 9.2% (Fig. 1e). This result is confirmed by the scanning electron micrographs of the particles with higher bSA loading, which show an extremely wrinkled appearance without polysorbate 80 (not shown) indicating the presence of more protein in the surface.

Their appearance is only marginally altered at $\mathbf{m_{s/p}} = 1.0$ with 1 mg/ml polysorbate 80 in the spray solution (not shown).

The ESCA-result for pure sodium dodecyl sulfate (SDS) shows, apart from the Na1s and Na2s peaks and their Auger peak at 502.4 eV, also the O1s, C1s, S2s and S2p peaks (Fig. 4a). The S2p peak at 180 eV can be used to determine the presence and relative concentration of SDS in the surface layer of the spray-dried trehalose particles, since it does not overlap with the C1s, O1s or N1s peaks of the trehalose or bSA. The measured % relative atomic concentrations of C, O, S and Na in the pure SDS (Table 1) compare closely with those calculated from the molecular structure of this molecule (66.7%, 22.2%, 5.6% and 5.6% for $C_{12}/O_4/S_1/Na_1$). Up to $\mathbf{m_{s/p}} = 5$ ($\equiv$ 0.1 mg/ml SDS in spray solution) there is no reduction in the % relative atomic concentration of N within the surface layer of the spray-dried trehalose/bSA particles (Fig. 4b). The % relative atomic concentration of C increases and of O decreases up to $\mathbf{m_{s/p}} = 5$ (Fig. 4c), which again may be a result of surfactant displacing trehalose from the water/air interface of the atomized spray droplets up to t_{crit}. There is, however, no change in % relative atomic concentration of S from the S2p peak (Fig. 4b), as must occur in the presence of SDS. Increase in $\mathbf{m_{s/p}}$ from 5.0 up to 47.5 causes a reduction in the % relative atomic concentration of N in the surface layer to zero (Fig. 4b). Simultaneously there is an increase in both the S2s and Na1s peaks, which can only come from the presence of SDS. It follows that the concentration-dependent exclusion of bSA from the solid surface (decrease in N1s) runs hand-in-hand with an increase in the surface concentration of the surfactant (increase in S2s). As already observed with polysorbate 80, however, the surface is not fully covered with SDS at high $\mathbf{m_{s/p}}$ where N1s is zero. Thus at $\mathbf{m_{s/p}} = 47.5$ the solid surface contains only approx. 40–50% SDS according to both the S2p, Na1s (Fig. 4b) and the C1s, O1s (Fig. 4c) results by comparison with those values for pure SDS and pure trehalose in Table 1. The absence of N1s means that the remainder of the surface is occupied by non-surface-active trehalose rather than surface-active bSA, as found above with polysorbate 80. The simple picture of competitive adsorption of surfactant and bSA at the water/air-interface up to $\mathbf{t_{crit}}$ cannot therefore be correct, otherwise the solid surface would be fully covered with surfactant when N1s = 0. The complete exclusion of the bSA from the solid surface when only approx. 50% (for SDS) or 80% (for polysorbate 80) of the surface is occupied with surfactant implies that only a fraction of the bSA and surfactant molecules present in the bulk spray solution prior to atomization are free to adsorb to the water/air-interface. It is known that SDS and bSA form a complex in aqueous solution via ionic and hydrophobic interactions (19,20), leading to denaturation at concentrations above the CMC. Complex formation between non-ionic surfactants (such as polysorbate 80) and other proteins (lysozyme, gelatine) has also been demonstrated (19,21). Under these conditions the concentrations of free protein and free surfactant in the bulk solution and hence available to adsorb to the water/air-interface will be reduced. This could explain the ESCA results presented here. We suggest that protein exclusion from the water/air-interface of the atomized spray droplets is a result of entrapment within the bulk solution as a result of complex formation with the surfactant. The reason for the higher relative molar concentration of SDS ($\mathbf{m_{s/p}} = 5$) than of polysorbate 80 ($\mathbf{m_{s/p}} = 1.0$) necessary before the protein start to be excluded from the surface layer of the particles (cf. Figs. 2 and 4b) remains to investigated.

As seen already with polysorbate 80, the scanning electron micrographs of the SDS-containing particles correlate well with the ESCA results. Below $\mathbf{m_{s/p}} = 5$ the spray-dried particles retain their wrinkled appearance (Fig. 5a–c). At $\mathbf{m_{s/p}} = 5$–50 the spherical, smooth appearance seen with pure spray-dried trehalose gradually reappears (Fig. 5d & e). Again, changes in surface composition and surface morphology induced by surfactant are unequivocal.

The addition of liposomes made from Lipoid E80 to spray solutions containing 5 mg/ml bSA and 95 mg/ml trehalose is intended as a negative control, since their presence did not ameliorate the inactivation of another protein, LDH, induced during spray drying with trehalose (4). As seen in Fig. 6, there is no change in % relative atomic concentration of N in the surface of the spray-dried bSA/trehalose particles with up to $\mathbf{m_{s/p}} = 180$. The non-adsorbable liposomes cannot hinder adsorption of protein from the water/air-interface of the spray droplets by either complex formation or adsorption to the interface. The scanning electron micrographs (not shown) show no change in the wrinkled appearance of the particles up to $\mathbf{m_{s/p}} = 180$.

CONCLUSIONS

The ESCA results presented show that the addition of polysorbate 80 to a spray solution containing trehalose and bSA results in a concentration-dependent hindrance of bSA adsorption at the surface of the spray-dried particles. The result with sodium dodecyl sulfate illustrates that the surfactant concentration in the particle surface increases whilst that for bSA decreases. It is also possible to identify the relative molar ratio of surfactant: protein necessary to displace completely the protein: this is higher for SDS than for polysorbate 80. On complete displacement of bSA from the solid surface, the latter is, however, not fully occupied with surfactant, suggesting that the dissolved protein and surfactant within the spray solution is not fully available for adsorption to the water/air-interface. Protein exclusion from the water/air-interface could therefore be a result of complex formation between protein and surfactant

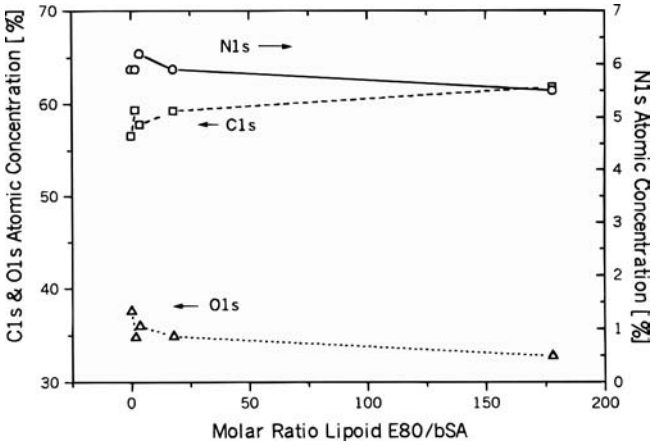

Fig. 6. Influence of molar ratio Lipoid E80/bovine serum albumin (bSA) ($\mathbf{m_{s/p}}$) on % relative atomic surface concentrations of N, C, and O in spray-dried trehalose/bSA. Spray solutions contained 5 mg/ml bSA and 95 mg/ml trehalose.

in the bulk spray solution prior to atomization. This information is surely useful when developing protein formulations for spray-drying.

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