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Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide

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Abstract

The micronization of various drugs is beset with serious problems due to the insufficient brittleness of crystals when using a jet mill. The purpose of this study was to investigate an alternative micronization technique using the aerosol solvent extraction system (ASES). Several steroids, some for systemic and some for administration by inhalation, were dissolved in an organic solvent and sprayed into supercritical carbon dioxide. The resulting particles were characterized with regard to chemical and physical properties. The following steroids were investigated: beclomethasone-17,21-dipropionate, betamethasone-17-valerate, budesonide, dexamethasone-21-acetate, flunisolide, fluticasone-17-propionate, prednisolone and triamcinolone acetonide. The spraying solution contained 1% (w/w) of drug, the solvents were dichloromethane, methanol or a mixture of both. The median particle size of the steroid particles was in most cases lower than 5 μm and consequently within the respirable range. If a surface active ingredient was added to the spraying solution the particle size increased and the contact angle decreased. HPLC-analysis showed no chemical decomposition of the drug during the process but the crystal properties of certain investigated drugs changed. This was proved by use of X-ray diffraction and scanning electron microscopy (SEM). Most of the steroids used could be micronized by means of the ASES-process with a residual dichloromethane content lower than 350 ppm in all cases.

Keywords: Aerosol solvent extraction system (ASES); Micronization; Pulmonary delivery; Steroids; Supercritical carbon dioxide

1. Introduction

Drug particles with a narrow particle size distribution, and a typical mass median aerodynamic diameter (MMAD) less than 5 μm are required for the application in inhalation therapy with a dry powder inhaler (DPI), a metered dose inhaler (MDI) or a nebuliser for an effective drug delivery to the lungs (Hinds, 1982). The micronization of drugs could be obtained by controlled crystalliza-

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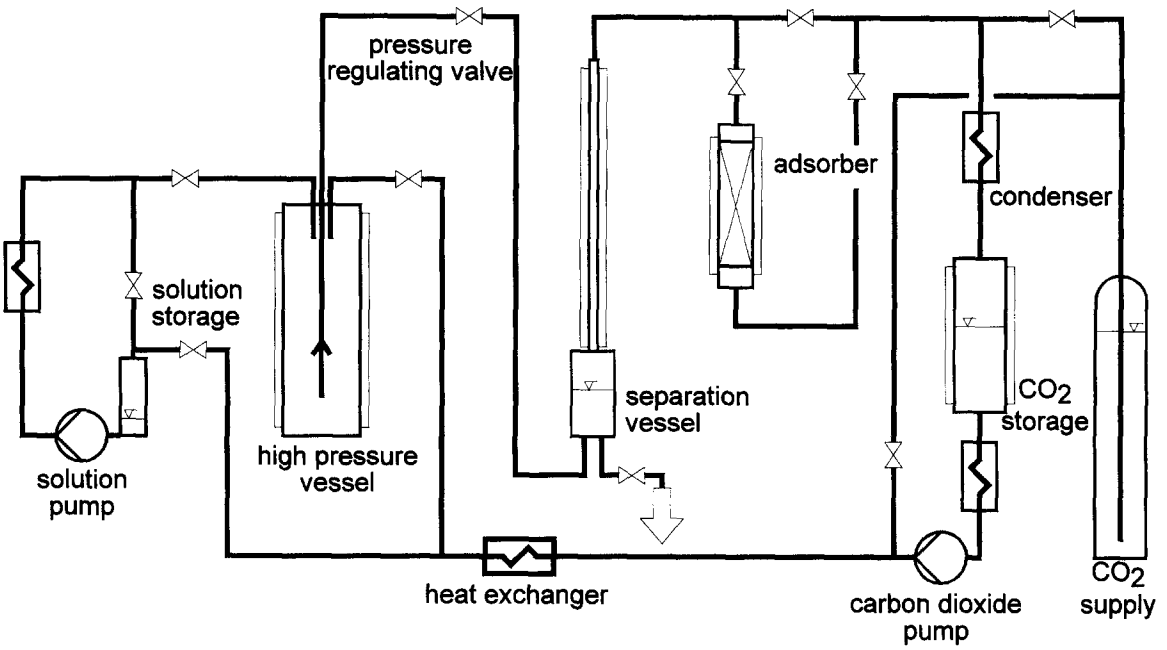


Fig. 1. Scheme of the ASES process.

tion out of organic solvents leading to solvent incorporation into the drug crystals (Gallagher et al., 1992). Jet milling of drugs is inappropriate or ineffective for unstable or wax-like substances particularly if they are unstable at higher temperature (Gallagher-Wetmore et al., 1994). Glucocorticoids for inhalation therapy of lung diseases are often very hydrophobic and have an insufficient wettability in water. With regard to the potential use in MDIs containing chlorofluorocarbon-free (CFC-free) propellants the production of microspheres coated with a surface active ingredient seems very promising because most of the widely used surfactants in MDI-formulations have poor solubility in the alternative propellants (Byron et al., 1994). In addition, particles with sufficient flow properties, low agglomeration tendency and

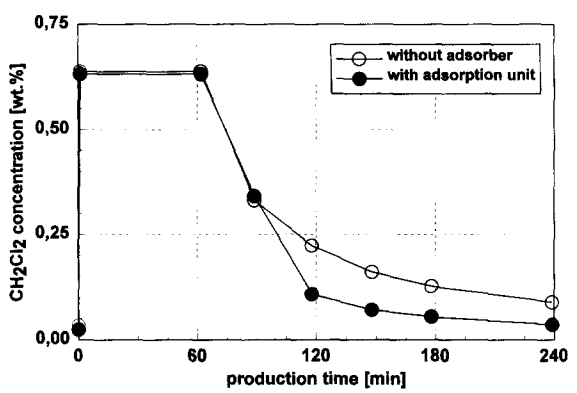
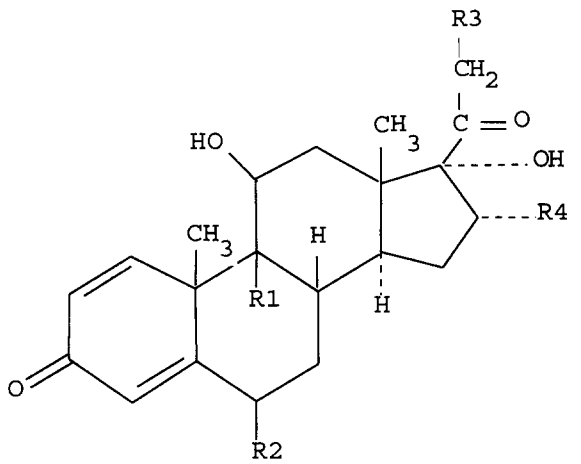


Fig. 2. Content of residual dichloromethane in the high pressure vessel versus production time.

Table 1
Experimental settings of the headspace gas chromatography

Experimental conditions	
Stripping unit	AMA LS-MA
Sorbent tube	Tenax TA at 30°C
Gas	Nitrogen, 200 ml
Desorption unit	AMA KA-D
Temperature	200°C for 5 min
Temperature cold trap	−120°C for 5 min
GC	Hewlett Packard 5880
Carrier gas	Helium at 1 bar
Detector	FID at 250°C
Column	Chrompack, 25 m × 0.32 mm coated with Poraplot Q
Oven temperature program	160°C for 10 min, 160–225°C with 10°C/min, 225°C for 30 min

Table 2
Chemical structure of the steroids



Steroid (manufacturer, country, batch-no.)	R1	R2	R3	R4	C16-C17-Connection	Used derivative
Budesonide (Farmabios, Italia, 0017427)	–H	–H	–OH	–OH	Butyronide	Butyronide
Triamcinolone (Diosynth OSS, Netherlands, 2044)	–F	–H	–OH	–OH	Acetonide	Acetonide
Flunisolide (Boehringer, Germany, EO 3318)	–H	–F	–OH	–OH	Acetonide	Acetonide
Prednisolone (Merck, Germany, K 22577)	–H	–H	–OH	–H	—	—
Fluticasone (Glaxo, Germany, WE 0971971)	–F	–F	–S–CH_3	–CH_3	—	-17-propionate
Betamethasone (Sicor, Italia, 4888/M1)	–F	–H	–OH	–CH_3	—	-17-valerate
Dexamethasone (Merck, Germany, K 10320)	–F	–H	–OH	–CH_3	—	-17-acetate
Beclomethasone (Roussel-Uclaf, France, 2 V 0604 BC)	–Cl	–H	–OH	–CH_3	—	-17,21 dipropionate

a batch to batch conformity are required for a good DPI-formulation (York, 1994). Bleich (1995) showed that the processing of poly-L-lactide with the addition of phosphatidylcholine (PC) in the aerosol solvent extraction system (ASES) led to improved wettability. An objective of this study was to prove whether these results were transferable to steroid particle production.

Furthermore different authors discussed the possibility of a sustained drug release from biodegradable isoproterenol loaded microspheres (polyglycolide-co-lactide) prepared by solvent evaporation (Lai et al., 1991). Boyes et al. (1988) produced biodegradable microspheres containing salbutamole or terbutaline using spray drying techniques.

Microspheres with the required properties can be produced using supercritical carbon dioxide. In principle two different production methods are possible: if the drug is soluble in an appropriate amount of supercritical CO_2 the fluid can be expanded rapidly into a subcritical gasphase and the drug precipitates (Stahl and Glatz, 1984; Loth and Hemgesberg, 1986; Tom and Debenedetti, 1991). Drugs which are insoluble in supercritical carbon dioxide can be dissolved in an organic solvent and when this solution is sprayed into the supercritical CO_2-phase the organic solvent is extracted while the drug precipitates (Müller and Fischer, 1989; Bleich et al., 1993).

2. Materials and methods

2.1. Microparticle production

The microparticle production is illustrated in the diagram in Fig. 1. A solution of steroid in

dichloromethane was sprayed by means of a diaphragm pump (solution pump) through a nozzle into the high pressure vessel filled with supercritical carbon dioxide. The organic solvent is soluble in the supercritical gas phase and will be extracted while the insoluble drug precipitates. The particle formation at the adjusted extraction conditions can be described as a precipitation and not as a spray drying process (Dixon et al., 1993). The experiments were carried out with a steroid concentration of 1% by weight and a pump rate of 63 ml/min. A nozzle (Schlick GmbH, Coburg, Germany) with a diameter of 0.3 mm and a spraying angle of 30° was applied.

Carbon dioxide was pumped with a diaphragm pump (CO_2 pump) into the high pressure vessel where temperature and pressure were kept constant at 40°C and 8.5 MPa during the extraction process. In the first hour after the spraying process the carbon dioxide pump was switched off to allow the steroid particles to sediment. The sedimentation phase does not influence the particle formation, but increases the yield of product (Ruchatz, 1996).

After this period of time the carbon dioxide pump was switched on again and the gas was expanded at the downstream side of the high pressure vessel to subcritical conditions using the pressure regulating valve. This expansion leads to a decrease in temperature of the carbon dioxide and implies the coexistence of gas and liquid phase. Due to the thermodynamic equilibrium the concentration of organic solvent in the liquid phase is distinctly higher than in the gas phase (Prausnitz, 1986). By removing the liquid phase in intervals out of the separation vessel the amount of organic solvent in the apparatus was rapidly reduced. Additionally an adsorber filled with active carbon can be used for a more effective removal of residual solvent in the carbon dioxide stream. Fig. 2 shows the decrease of dichloromethane concentration versus production time in the high pressure vessel as an example for two produced batches with and without the use of the adsorption unit. The carbon dioxide is then condensed and stored in the CO_2-storage vessel.

The microparticles were dried by keeping them in the supercritical carbon dioxide for a total production time of 3 h. All experiments were carried out twice.

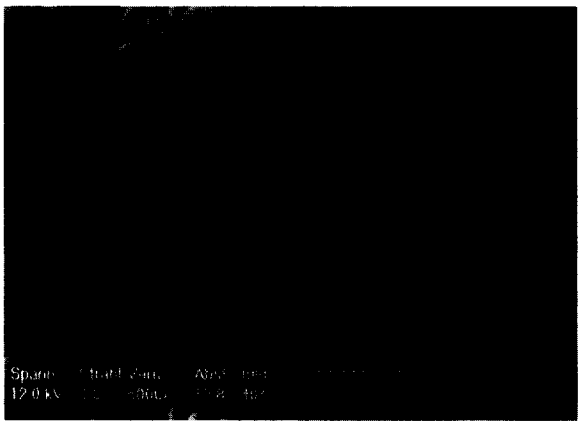


Fig. 3. SEM photograph of budesonide produced with ASES without PC.

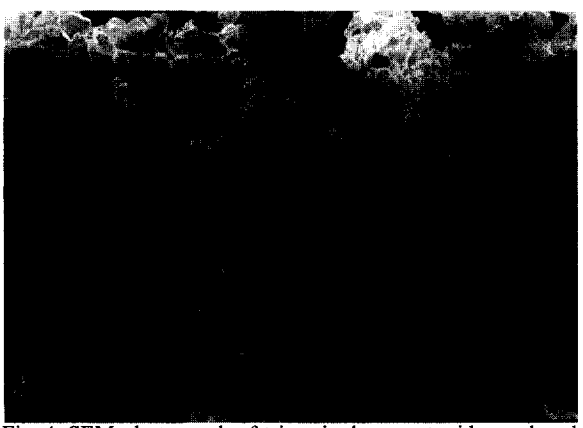


Fig. 4. SEM photograph of triamcinolone acetonide produced with ASES without PC.

2.2. Characterization methods

2.2.1. Particle size

The volume particle size distribution of the microspheres was determined using a laser diffractometer (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). The particles were suspended in an aqueous solution of polyoxyethylene sorbitane monooleate (0.01% per weight). The particle size distribution was measured before and after 90 s of ultrasonication treatment. Previous test series have shown that the deagglomeration process was completed after 90 s. This is in agreement with

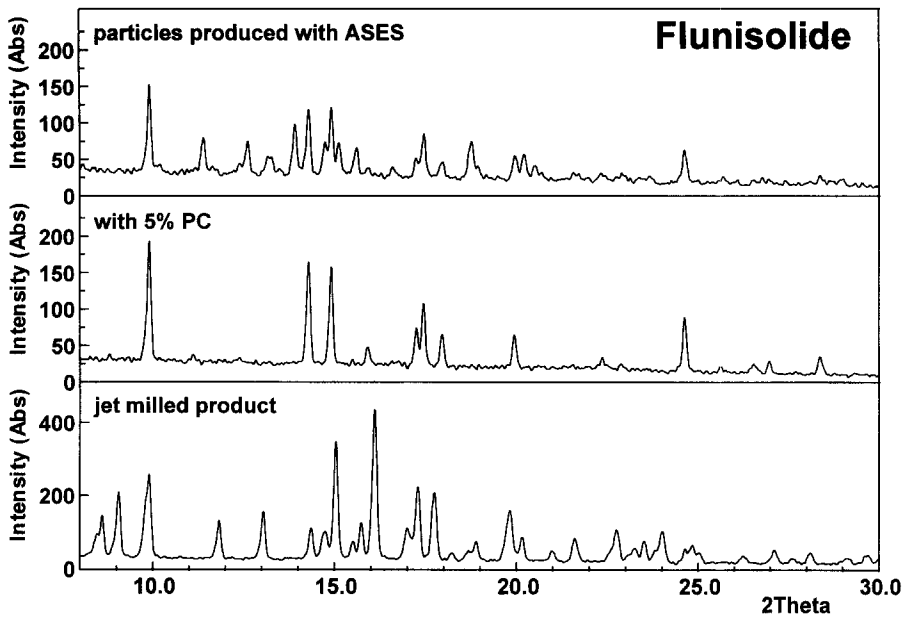


Fig. 5. X-ray patterns of flunisolide.

Fig. 6. (a) SEM photograph of Flunisolide produced with ASES without PC, (b) with phosphatidylcholine.

results from (Bleich et al., 1994). The ratio of median particle size (× 50%) before ultrasonication to median particle size after ultrasonication was calculated and then termed 'index of agglomeration'.

2.2.2. Determination of crystallinity

To determine the crystallinity patterns of the micronized steroid batches an X-ray powder diffraction system (Stoe and Cie GmbH, Darmstadt, Germany) with a rotating anode was used. The measuring unit consists of a rotating anode in transmission technique and with the following specifications: Cu $K\alpha_1$, Radiation, Graphite Monochromator, Voltage: 40 kV, current: 200 mA, Position Scanning Detector (PSC), scanning rate of 10 s/2θ over a range of 5–50° 2θ.

2.2.3. Particle morphology

The particle morphology was performed by SEM. Photographs were taken by a Philips XL 20

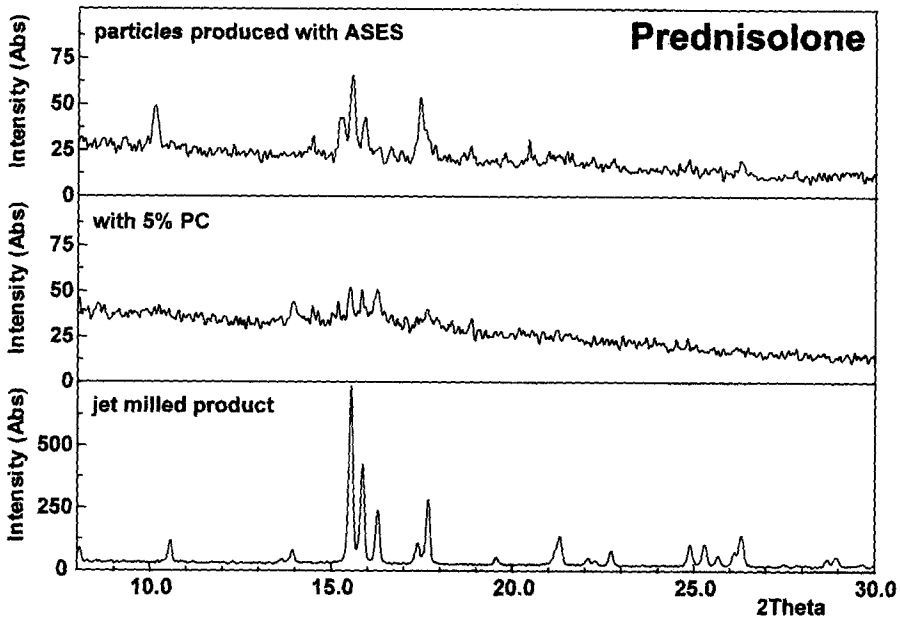


Fig. 7. X-ray patterns of prednisolone.

(Philips, Eindhoven, Netherlands). The particles were fixed on a mutual conductive adhesive tape (Leittabs, Plano, Marburg, Germany) on an aluminium sample disk. These samples were sputtered with gold for three minutes choosing a current of 50 mA under argon atmosphere at 0.05 Pa using a Sputter Coater SCD 005 (Balzas Union, Balzas, Liechtenstein).

Fig. 8. SEM photograph of prednisolone produced with ASES without PC.

2.2.4. High pressure liquid chromatography

The HPLC system consisted of a Gynkotek High Precision Pump Model 300 (Gynkotek, Munich, Germany), a Kontron HPLC Autosampler 360 (Kontron Instruments, Milano, Italy), a Shimadzu UV spectrophotometric detector, a Shimadzu Chromatopak C-R 3A Integrator (Shimadzu, Kyoto, Japan) and LiChrospher 100 RP18 columns (4.0 × 125 mm) obtained from Merck (Darmstadt, Germany). Samples of 100 μl were injected. As mobile phase acetonitrile/water-mixtures were used. The flow rate was 1.2 ml/min resulting in a pressure of about 8.0–9.5 MPa. The amount of drug was calculated using an external standard.

2.2.5. Contact angle measurement

The contact angle was determined using the Erma Contact Angle Meter G-1 (Erma Optical Works, Tokyo, Japan). By application of a single drop of water (25 μl) on a tablet consisting of about 50 mg of drug which was compressed at 25 kN for 2 min under vacuum, the contact angle was defined twenty times for each sample.

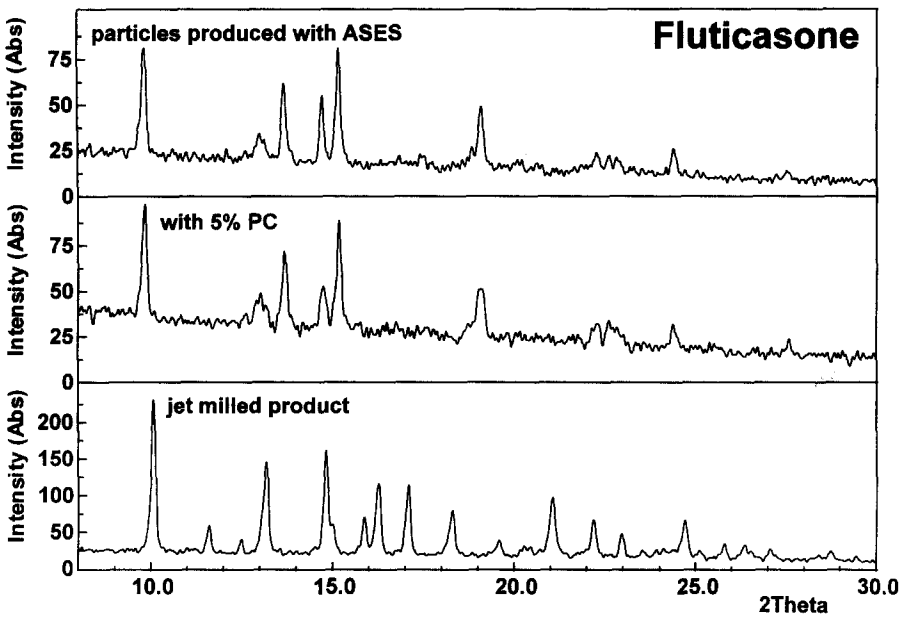


Fig. 9. X-ray patterns of fluticasone.

2.2.6. Dichloromethane content in the CO_2 stream

For the determination of dichloromethane in the circulating gas stream of the ASES an aliquot of the CO_2 stream was filled in a sampling tube after different periods of time. A volume of 1000 μl was directly injected into a gaschromatograph (Varian 370, Varian Instruments Group, Palo Alto, USA) equipped with an FID detector. The content of residual dichloromethane was calculated using an external standard.

2.2.7. Dichloromethane content in the drug particles

Headspace gas chromatography was used to determine the residual content of dichlormethane. 25 mg of steroid microparticles were dissolved in 0.5 ml dimethylacetate. Then 0.5 ml of water containing the internal standard diisopropylether was added to the solution. The resulting suspensions were stripped with nitrogen. The extracted volatiles were enriched in an adsorptive process on a column filled with Tenax TA. After thermal desorption of the column and concentration in a cool trap the chromatographic system was started by heating the trap (Ruchatz, 1996). The experimental conditions are listed in Table 1.

2.3. Materials

The steroids listed in Table 2 were used for the experiments. All of them were of pharmaceutical grade with a content of active ingredient greater than 99%. For the dissolution of drug dichloromethane and methanol in analytical grade (Merck KG, Darmstadt, Germany) were employed. The experiments with the surface active ingredient were performed with Lipoid E 80-3 (Lipoid KG, Ludwigshafen, Germany, Batch-No. T 99510-2). The used carbon dioxide was of high quality (99.97%), supplied from Messer-Griesheim, Frankfurt, Germany.

3. Results and discussion

Micronizing steroids in an inert medium leads to a pure product while the milling of drugs is often afflicted with an increase of impurities like metal abrasion. During the ASES-process a micronization and a coating with a surfacant or other hydrophilizing agents was achieved within one process step. Using PC as physiological surfactant which is widely employed in parenteral fat

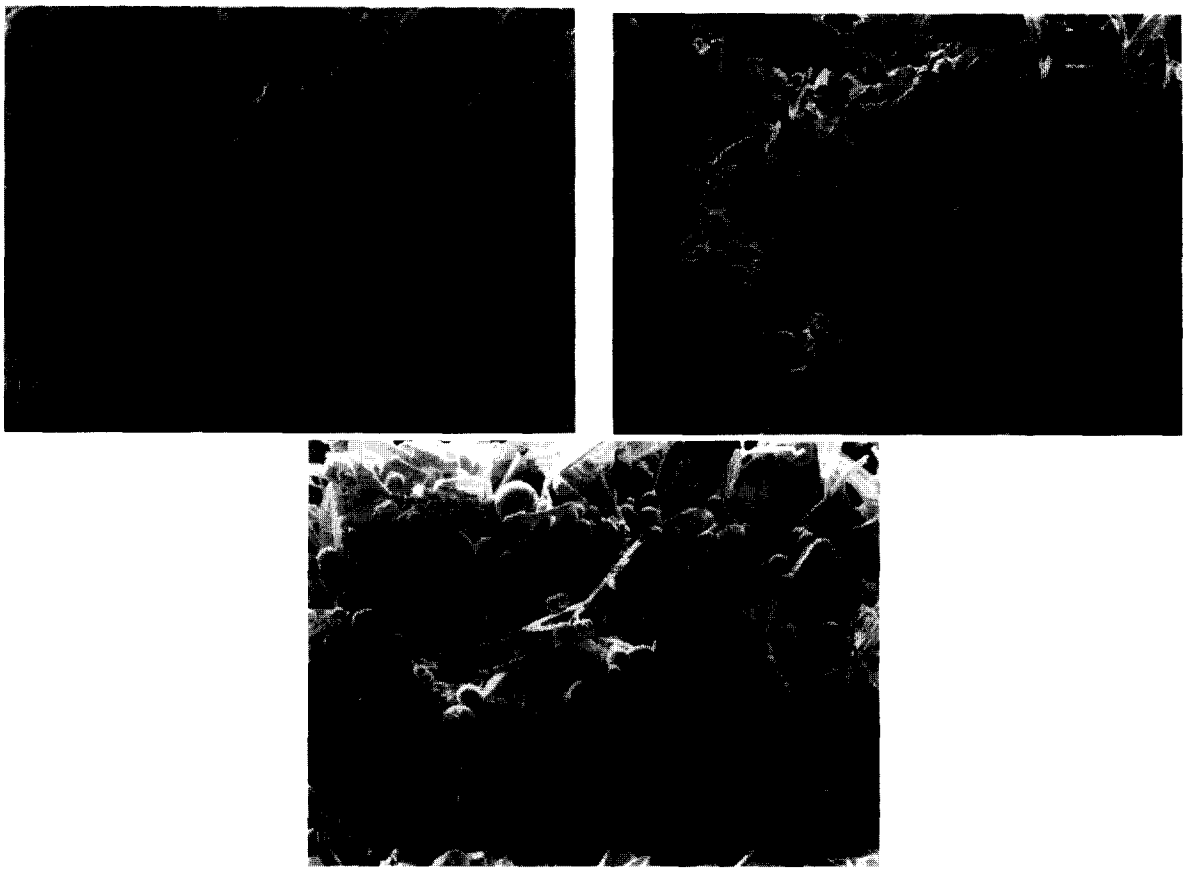


Fig. 10. (a) ASES photograph of jet milled fluticasone, (b) fluticasone-17-propionate produced with ASES without PC and (c) with phosphatidylcholine.

emulsions is not expected to cause irritations within the respiratory tract (Müller and Iacono, 1967).

One requirement for the use of this device is that the drug does not decompose during the micronization process. By comparing the HPLC-chromatograms before and after processing in the ASES-apparatus we concluded that the quality of the drugs did not change.

The X-ray-diffraction measurements led to different results. Budesonide showed no change in crystallinity neither with the addition nor without the addition of PC to the spraying solution. When evaluating the SEM-photograph no differences in morphology of the particles were observed. The resulting particles were nearly spherical and non-porous (Fig. 3).

With triamcinolone acetonide (TCA) no differences in crystal modification produced with and without PC and the jet milled drug occurred. These results are in agreement with the investigations of Mesley (1972), who did not discover polymorphism. However, a high loss in peak intensity of the X-ray diffractograms can be observed. This indicates a decrease in crystallinity. Fig. 4 illustrates TCA-microspheres sprayed without addition of the surfactant. The PC containing particles showed the same morphology.

Both micronized batches of flunisolide (with and without PC) using supercritical carbon dioxide showed a decreased X-ray peak intensity (Fig. 5). The X-ray patterns of both batches of ASES-particles have changed in comparison with the jet milled product indicating polymorphism. The par-

Table 3
Partition coefficients of the steroids (modified from Hansch et al., 1990)

Steroid	log P (Experimental)	log P (Calculated)	Particle formation
Beclomethasone-17,21,-dipropionate	3.60	4.30	–
Betamethasone-17-valerate	–	4.2	–
Budesonide	–	2.31	+
Dexamethasone-21-acetate	2.91	2.7	Film
Flunisolide	–	1.7	+
Fluticasone-17-propionate	–	3.9	+
Prednisolone	1.62	1.6	+
Triamcinolone acetonide	2.53	2.7	+

–, no particle formation, +, particle formation.

ticles produced with supercritical carbon dioxide showed considerable differences in particle shape (Fig. 6a,b).

The produced batches of prednisolone showed a decrease in X-ray peak intensity of both ASES-products compared to the jet milled drug. The particles produced by means of ASES without PC showed another modification than the jet milled product. The batches with PC led to a nearly amorphous powder (Fig. 7). However, the morphology of the ASES-particles did not differ distinctly. A SEM-photograph of the surfactant-free particles is shown in Fig. 8.

After the ASES-process the X-ray-diffractogram of the fluticasone-17-propionate particles showed a different crystal modification as compared to the jet milled product (Fig. 9). Furthermore the peak intensity decreased. The fluticasone particles with and without addition of phosphatidylcholine resulted in equal X-ray patterns. The SEM study confirmed this observation. The ASES-products showed a similar particle morphology in contrast to the jet milled drug (Fig. 10a,b,c). Either the precipitated fluticasone appears in two different habits or the spherical forms are amorphous while the ribbons exhibit a crystal drug modification.

Table 4
Residual dichloromethane content of the steroid microparticles

Steroid	Residual solvent content (ppm)	
	Without PC	With 5% PC
Budesonide	302	26
Flunisolide	81	142
Fluticasone	18	243
Prednisolone	51	211
Triamcinoloneacetonide	33	51

With the other drugs (betamethasone-17-valerate, dexamethasone-21-acetate and beclometasone - 17,21 - dipropionate) no particles were obtained. This may be due to their higher partition coefficient (Table 3) calculated for the octanol/water system (Hansch et al., 1990). Stahl and Glatz (1984) found that steroids have better solubility in supercritical carbon dioxide with increasing hydrophobicity (Loth and Hemgesberg, 1986). The used glucocorticoids with a logarithm of the partition coefficient (log P) lower than approximately four are not extractable from supercritical carbon dioxide under the adjusted production conditions. These drugs led to particle formation with the exception of dexamethasone-21-acetate. The processing of this drug resulted in a film adhering to the inner sides of the high pressure vessel. Betamethasone-17-valerate and beclomethasone-17,21-dipropionate were extracted by supercritical carbon dioxide under the applied conditions.

The extraction of the solvent dichloromethane is highly dependent on the production time (Fig. 2). The residual solvent content of microparticles was found to be lower than 350 ppm in all cases; most of the experiments yielded a residual solvent content of lower than 250 ppm as shown in Table 4. The dissonances of the residual

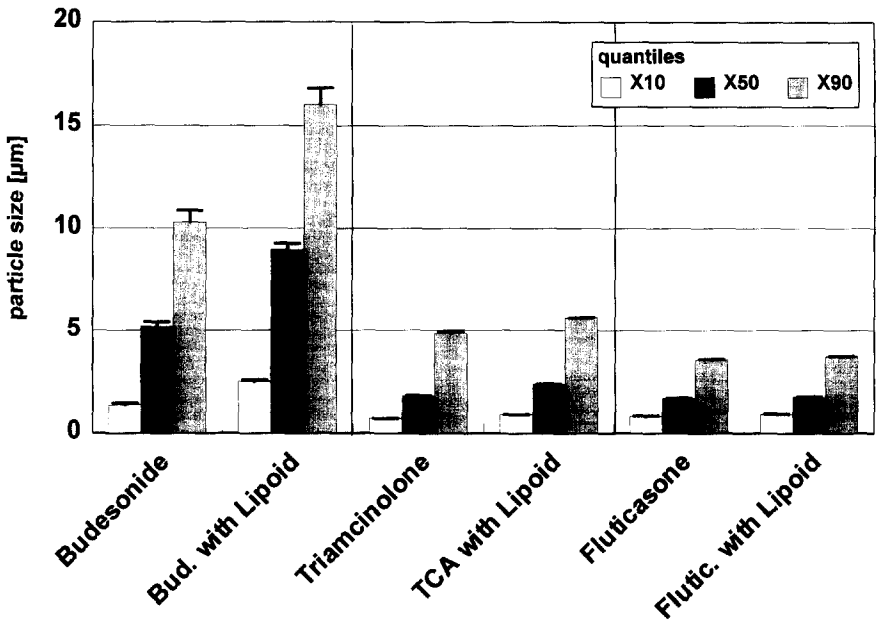


Fig. 11. Particle size of microspheres produced by means of ASES.

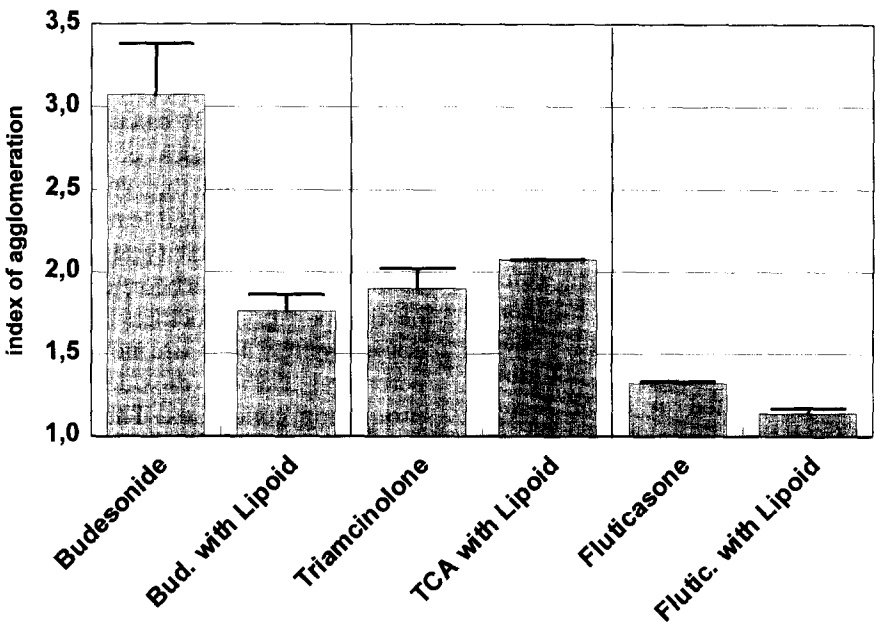


Fig. 12. Index of agglomeration of the ASES particles.

dichloromethane content of particles produced with and without PC could not be explained.

Due to their possible application in inhalation therapy the particle size is of great importance: The median particle size was found to be less than 5 μm in all experiments with the exception of budesonide sprayed with PC (Fig. 11). The particle size distribution of flunisolide and prednisolone could not be determined because of their partial solubility in water. However, SEM-photographs indicated that the particle size was in the same range (Fig. 6a,b and Fig. 8).

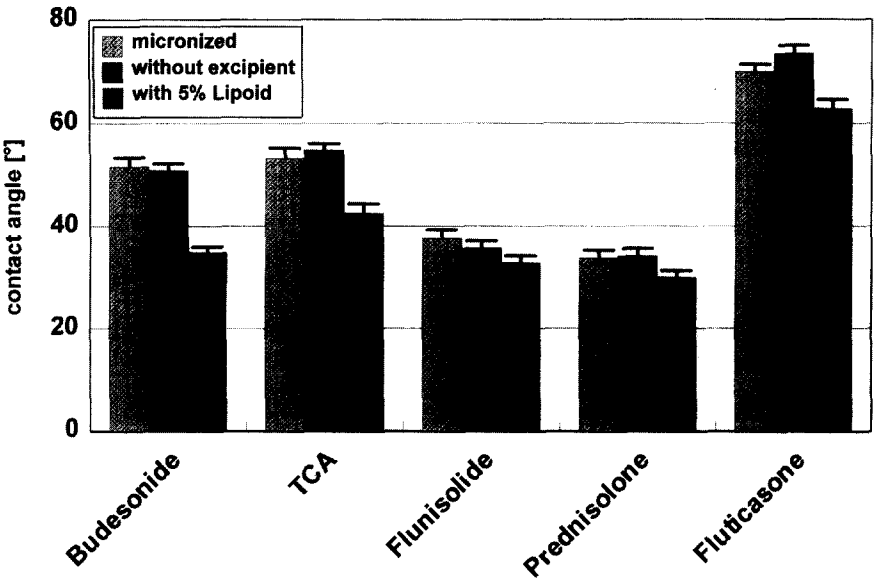


Fig. 13. Contact angle measurements.

Obviously the particle size increased if phosphatidylcholine was added to the spraying solution. This could not be explained with a higher agglomeration tendency (Fig. 12) because the index of agglomeration did not increase with addition of PC. An influence of the PC on the precipitation process was presumed. The consideration of the X90- and X10%-values of particle size distribution showed that the produced particles are suitable for an inhalation therapy (except budesonide plus PC).

With the addition of phosphatidylcholine a remarkable decrease of the contact angle was reached (Fig. 13). This could be advantageous for suspensions of the drug particles in water, hydrophilic solvents or propellants.

4. Conclusions

The presented study showed that a micronization of several steroids could be achieved by means of the ASES. It has been proved that no chemical decomposition took place during the treatment with supercritical carbon dioxide. The aim to produce particles in the respirable range was obtained. With addition of phosphatidylcholine the particle size slightly decreased but the wettability increased.

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