PROCESS AND PRODUCT CONTROL OF POROUS MICROPARTICLES – A LONG ACTING RELEASE FORMULATION

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ABSTRACT

The porous structure of microparticles strongly influences the drug release profile of this parenteral long acting release formulation. An ultrasound extinction spectroscopy method was developed for on-line monitoring the primary emulsion process step, which in turn defines the porosity of the microparticles. Experiments were performed showing the influence of the dispersion conditions on the emulsification and the feasibility of scale up. Different bulk powders of microparticles were investigated by mercury porosimetry and nitrogen gas sorption. Both methods are sensitive to the porosity. Furthermore a combination of these data with particle size distribution data is suggested, which allows a quantitative evaluation of a mean pore diameter for spherical particles with spherical pores, as obtained by the W/O/W double emulsion process.

1 INTRODUCTION

Injectables are widely used pharmaceutical delivery forms, especially for proteins, peptides or other water soluble drugs. These therapeutics have a relative rapid clearance from the bloodstream. If a treatment over a longer time period is necessary, the patients require multiple injections in order to ensure a certain drug level. In such cases a long acting, controlled release of a drug substance is highly desirable. This can be achieved by embedding the drug substance into a polymer matrix made out of biodegradable polymers, e.g. Poly(lactide-co-glycolide). To encapsulate these water-soluble drug substances the water-in-oil-in-water (W/O/W) double emulsion microparticle preparation process has been established during the past decade [1]. Due to this two step emulsification process the final microparticles are highly porous and the release of drug substance is strongly dependent on this porous structure. The critical pore forming process step is the preparation of the primary W/O emulsion. Thus, in order to control drug product quality, drug release profile and batch to batch reproducibility, there is a need for on-line monitoring of the W/O emulsification process. This holds especially during development, were scale up experiments from small lab scale to production scale are performed. For this purpose ultrasound extinction spectroscopy (USE) was evaluated [2] and applied to scale up experiments covering a scale up factor of 10.

In addition to process monitoring there is also a need for suitable analytical methods to characterize the porosity of the final microparticles. Scanning electron microscopy (SEM) on fractured microparticles can be used to get a qualitative impression about morphology and size of the pores. However this method is very time consuming and only few particles can be analyzed, thus representative sampling may cause problems. Mercury porosimetry is a well established analytical method to measure powder porosity. However in the case of the microparticles the main assumption i.e. cylindrical pores, is not fulfilled. This makes the applicability of this method questionable. As alternative method nitrogen gas sorption may be applied. But the typical pore size is in the range up to tens of micrometers. Therefore this method is also not applicable for a quantitative pore size analysis [3]. Nevertheless microparticles with distinct porosities were manufactured and analyzed with these two classical analytical methods and the applicability of these methods was evaluated.

2 MATERIALS AND METHODS

Poly(lactide-co-glycolide) W/O/W microparticles were prepared similarly as described in [1]. For the W/O primary emulsion process a gear pump was used as dispersion device and a static mixer for the secondary emulsion process.

For on-line monitoring of the W/O process an ultrasound extinction spectroscopy (USE) apparatus OPUS Sympatec was installed in a close loop setup. The USE attenuation spectra were recorded in the range of 0.2 MHz – 40 MHz. The droplet size distributions were obtained by transformation of the USE attenuation spectra according to an emulsion model.

Scanning electron microscopy (SEM) was performed with JSM6460 LV Jeol. For analysis of fractures the microparticles were embedded into an epoxy resin and fractured in fluid nitrogen.

Mercury porosimetry data were recorded with POREMASTER 60 GT Quantachrome.

BET specific surface area was investigated by Nitrogen

and Krypton gas sorption with AUTOSORB 3 Quantachrome

Particle size distributions were analyzed by laser light diffraction (LLD) using HELOS / CUVETTE Sympatec. Beside characteristic particle sizes the specific surface area by volume was evaluated.

Solid state density, or true density was measured by helium pycnometry, ULTRA PYCNOMETER 1000T Quantachrome.

3 RESULTS AND DISCUSSION

3.1 On-line Monitoring of the W/O Emulsion Process

Figure 1 shows USE on-line monitoring data of a typical lab-scale W/O emulsion process over a time period of two hours.
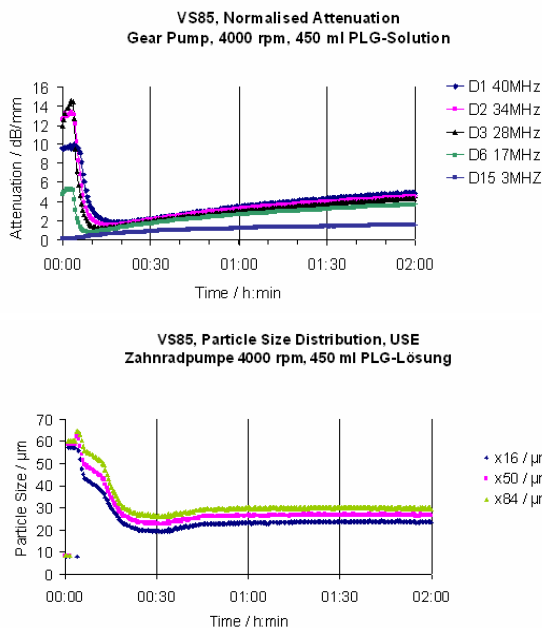

Figure 1: USE on-line monitoring of W/O emulsion process. Top: Time course of attenuation at five characteristic frequencies within 3 MHz and 40 MHz. Bottom: Corresponding time course of characteristic particle size values (x_{16}, x_{50}, x_{84}).

As indicated by the plots in figure 1 the gear pump dispersed the water phase down to an endpoint within the first 20 min (phase I). In the following the droplet size remained almost constant, whereas the attenuation spectra increased continuously (phase II). Similar data were also obtained using ultra turrax and ultrasound processor as alternative dispersion devices. The three dispersion devices could be distinguished in terms of kinetics and attenuation amplitudes. This

yields a rank order with respect to dispersion intensities: Weak for gear pump, middle for ultra turrax and strong for ultrasound processor.

To investigate the scale up of this process step a loop reactor was used for emulsification, equipped with a gear pump. The experiments were performed using different sample volumes in the range of 0.4 l – 4 l. In figure 2 the time course of several emulsification preparations are plotted as attenuation at 40 MHz versus number of turnovers of the sample volume used.
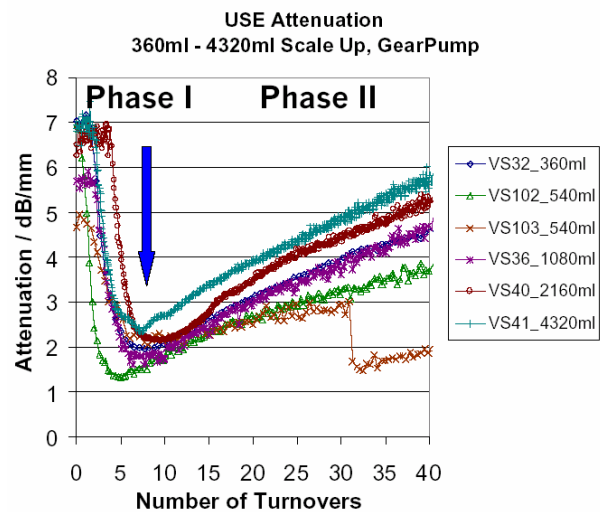

Figure 2: Plot of attenuation signals at 40 MHz obtained by USE during scale up experiments from 0.4 l to 4 l. The time axis (abscissa) is scaled to number of turnovers for ease of comparison between the different process volume sizes.

Throughout the different process volumes investigated, the attenuation signals changed in the same manner with increasing number of turnovers, thus forming a bundle of curves. The minimum attenuation after phase I was 2.0 dB/mm (STD_{rel} = 20%) and the "time" until minimum was 8.3 turnovers (STD_{rel} = 30%). The reason for the unexpected increasing attenuation in phase II is under investigation. According to these results the process was fairly robust and not sensitive to process time. Thus scale up to bigger volumes can be easily performed by increasing process time, while keeping dispersion intensity and flow rate constant.

3.2 Characterization of the Porous Structure of W/O/W Microparticles

The final microparticles are of spherical shape with an outer diameter of about 100 μm. The microparticles are highly porous. The diameter of the pores is in the range of 1 μm up to 20 μm or even more, depending

on process conditions. Figure 3 shows SEM pictures of a typical sample.
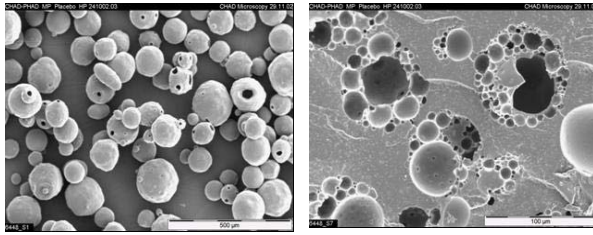
Figure 3: SEM pictures of spherical microparticles (left) and of a fracture (right) showing the inner porous structure of several individual microparticles

Microparticles of four different porosity classes were prepared and analyzed: (1) microparticles with mainly small pores, (2) with small as well as large pores, thus exhibiting a broad pore size distribution, (3) with mainly large pores and (4) with a dense structure. Mercury intrusion data of these samples are shown in figure 4.
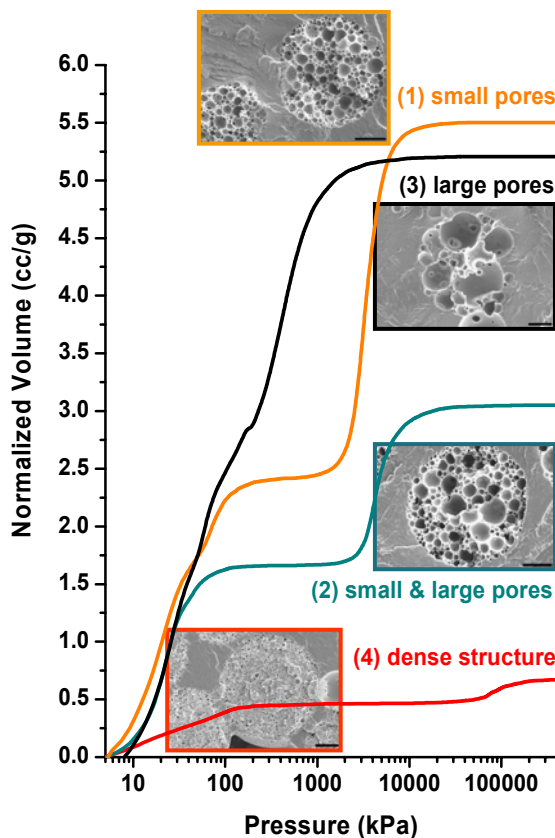
Figure 4: Mercury intrusion data of the different microparticle samples, (1) (2) (3) (4) see text. Due to the horizontal course between low and high pressure region, the inter- and intra-particle volume can be well separated. For comparison the SEM pictures show fractures of the corresponding microparticles of the different qualities.

The intruded volume in the low pressure range can be interpreted as filling of inter-particle volumes. The high pressure range above 1000 kPa for sample (1), (2) and (4) is characteristic for the intra-particle volume. According to this model and the distinct separation of the two regions, it is possible to calculate the total inner pore volume of the microparticles, which was in the range of 0.2 cm^3/g $\leq$ Vol_intra $\leq$ 3.0 cm^3/g.

The samples were also investigated by nitrogen gas sorption. Ad- and desorption isotherms were recorded over the full p/p_0-range, but could not be evaluated in terms of porosity, as already expected from the size of the pores. However the specific surface area according to BET could be obtained. The values of the four samples were in the range of 0.1 m^2/g $\leq$ Sm_BET $\leq$ 2.3 m^2/g and the different particle qualities could be well distinguished. Thus specific surface area measurements provided a second sensitive method to characterize these particles. In addition particle size distributions of the samples were obtained by laser light diffraction (LLD) measurements. The particle size distributions were similar for all 4 samples, with median values in the range of 80 µm $\leq$ x_{50} $\leq$ 120 µm. Furthermore the solid state density of these samples was measured, yielding ρ_s = 1.4 g/cm^3 for all four samples.

All these methods together provide a sound basis for a quantitative characterization of the size and structure of microparticles. However there is no single method which can provide a measure for the pivotal characteristic of these particles, the pore size. This can be achieved by combining the specific surface area data obtained by gas adsorption and particle size analysis, together with the specific intra-particle volume and the solid state density. Assuming spherical particles with spherical pores, the pore size can be evaluated according to the following equation:

$$D\_pore = 6 / (Sm\_BET / (Vol\_intra + 1/\rho_s) - Sv\_LLD)$$

with

D_pore	-	pore diameter [µm]
Sm_BET	-	specific surface area by mass, BET [m^2/g]
Vol_intra	-	specific intra-particle volume, Hg-porosimetry [cm^3/g]
ρ_s	-	solid state density, He-pycnometer [g/cm^3]
Sv_LLD	-	specific surface area by volume, LLD [m^2/cm^3]

Applying this equation to the data set yielded the following pore sizes of the microparticles: 11 µm for (1), 12 µm for (2) and 50 µm for (3). For sample (4) no meaningful value could be given as the low BET

specific surface area was very close to the outer surface area given by the particle size. The obtained pore size values were in agreement with SEM pictures of particle fractures thus demonstrating the applicability of this approach.

	Microparticles			
	(1) small pores	(2) small & large pores	(3) large pores	(4) dense structure
x_{50} [µm]	90	120	85	85
Sv_LLD [m^2/cm^3]	0.08	0.06	0.09	0.09
Sm_BET [m^2/g]	2.3	1.2	0.7	0.08*
Vol_intra [cm^3/g]	3.0	1.4	2.4	0.2
D_pore [µm]	11	12	50	--

* Measured by Kr-adsorption

Table 1: Characteristic values of the four microparticle preparations measured by laser light diffraction (x_{50}, Sv_LLD), BET gas sorption (Sm_BET), mercury porosimetry (Vol_intra) and the calculated mean pore size (D_pore).

The measured data and the calculated pore size values of the four samples are summarized in table 1. The data indicate that e.g. a similar pore size can be achieved within a wide range of pore volumes if specific surface area changes commensurately, or similar pore volumes can be achieved with different pore sizes.

Combining different methods to one value also combines the errors of each of the methods, which makes an impact on the precision of the pore size values. Therefore one has to take into account that this combination may not yield very exact values and of course cannot yield pore size distributions. However it eases interpreting data of different methods and thus may help to get a better understanding for process, structure and properties of the final microparticles.

4 CONCLUSION

Ultrasound extinction spectroscopy has been successfully applied for on-line monitoring of the primary W/O emulsion process. The experiments showed that the emulsion process yielded stable water droplets with minimal size, was robust and not sensitive to process time. Due to the stability with time, which is not obvious for emulsions, a scale up of a

factor of ten was achieved by adapting process time to process volume. However the attenuation spectra also revealed that a final equilibrium state could not be achieved. Thus on-line monitoring of the process is necessary, not only for scale up purposes but also for process steering, in order to control batch to batch reproducibility and quality.

Despite the initial doubts about the applicability of mercury porosimetry and gas adsorption to characterize these highly porous microparticles, both methods turned out to be sensitive to the porous structure. However each single method alone cannot yield the pore size. A combination of these data together with particle size and density measurements is suggested, which allows a quantitative evaluation of a mean pore diameter. The obtained pore size values may not only be used for quality control purposes. They may also help to optimize process conditions and to get a better understanding of the relation between pore size and drug release profile.

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