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New insights into the pore structure of poly(D,L-lactide-co-glycolide) microspheres

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ABSTRACT

The objective of this work was to develop a fast and significant method for the determination of the intraparticulate pore size distribution of microspheres. Poly(lactide-co-glycolide) (PLGA) microspheres prepared with a solvent extraction/evaporation process were studied. From the envelope and the skeletal volume of the microspheres the porosity was calculated. The skeletal volume was determined with nitrogen and helium pycnometry and mercury intrusion porosimetry. Based on single particle optical sensing (SPOS) a novel method was developed by which the envelope volume is calculated from the particle size distribution (PSD), provided that all particles have a spherical shape. The penetration capacity of the applied intrusion media is limited by their atomic or molecular diameter or by the surface tension and the pressure in case of mercury. A classification of the pore structure was obtained by comparing these different skeletal values with the values for the envelope volume. Two well separated pore fractions were found, a nanoporous fraction smaller than 0.36 nm and a macroporous fraction larger than 3.9 μm. The total porosity and the ratio between both fractions is controlled by the preparation process and was shown to depend on the solvent extraction temperature.

1. Introduction

Porous materials have found widespread use in many pharmaceutical and also technical applications, such as ion exchangers, adsorbents, chromatographic packings, supports for heterogeneous catalysis or solid-phase synthesis. Because of rapid advances in controlled drug delivery and tremendous growth of fields like solid phase catalysis and separation science, research on these kinds of materials has experienced a considerable uptrend in recent years. In all these applications the pore texture of the material is a crucial factor for its functionality and has to be optimized for the intended purpose. In many applications, for example, a bimodal pore size distribution is desirable with a network of large pores providing the pathways for an efficient mass transport and small pores providing a large active surface (Zhang, 2005; Leofanti et al., 1998). Numerous porous materials are designed in the form of microspheres, frequently manufactured via emulsification or spray drying processes. In case of microspheres for pharmaceutical use, the porosity has significant influence for example on drug release (Mao et al., 2008; Ghaderi et al., 1996; Klose et al., 2006; Lemaire et al., 2003; Luan and Bodmeier, 2006; Batycky et al., 1997). In other applications of microspheres where the pore texture is sometimes considered not to be a principal feature, porosity is at least an important quality characteristic and its significance is often underestimated.

In general the porosity describes the fraction of voids in a given volume of a material. Depending on the size and type of pores included by the measurement different values can be derived (Webb, 2001a). This fact is also reflected in the existence of different definitions of porosity. The American Society for Testing and Materials (ASTM) defines it as "the ratio, usually expressed as a percentage, of the total volume of voids of a given porous medium to the total volume of the porous medium" (American Society of Testing and materials, 1994), whereas the British Standards Institution (BSI) describes it as "the ratio of open pores and voids to the envelope volume (British Standards Institution, 1991). Thus a given porosity value has to be interpreted in consideration of (i) the range limits of the measuring method and (ii) the inclusion or exclusion of open pores. Especially in case of dispersed solids the precise determination of the particles' envelope volume is a difficult and often unsolved problem. Many intrusion media even mercury under low pressure were found to fill not only the interparticular voids but to penetrate also into open pores to a certain extent. Various approaches have been made to overcome this problem e.g. subtraction of the interparticular volume of crushed nonporous glass beads from the total void volume of the porous sample particles, both measured by mercury intrusion (Tonnellier, 2008).

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Mercury intrusion porosimetry is a common method to determine the porosity of a material and provides also information about its pore size distribution (Webb, 2001b). Mercury is a non-wetting, non-reactive liquid, which will not penetrate into small pores until a certain pressure is applied. The relationship between the pressure and the pore size, into which mercury is able to intrude, is given by the Washburn equation. It permits to acquire data over a broad range of pore diameter up to 360 μm, but implies several problems as well. Besides the difficulty to distinguish between intra- and interparticulate porosity as aforementioned, the measurement requires a toxic substance, relatively large sample quantities and is time-consuming. Furthermore ink-bottle shaped pores and interconnected pores shift the pore size distribution to smaller pores and bias the results (Allen, 1997; Dees and Polderman, 1981).

Another technique to gain information about the porosity is nitrogen adsorption. Beyond the specific surface area of the sample further textural characteristics can be derived from adsorption–desorption isotherms of nitrogen at its boiling point. However, only the micro- and mesoporous range of the pore distribution is covered by this method. Further approaches to determine the porosity are the water saturation (Kate and Gokhale, 2006) and water evaporation technique (Krus et al., 1997). In these methods the sample is allowed to equilibrate with an excess of water. The total volume minus the amount of the not absorbed water reflects the volume of the pore space. If water is able to cause swelling of the sample, these methods do not allow to distinguish between permanent and temporary porosity.

Despite their widespread use all these techniques lack in accuracy and/or simplicity and are not able to distinguish between intraparticulate pores and interparticulate voids. As mentioned before, the porosity can be calculated from the envelope and the skeletal density of the material. Gaspycnometry is an accurate method to determine the latter parameter, whereas the measurement of the apparent density is often difficult in case of a dispersed material. For this reason we developed a novel method for the determination of the intraparticulate porosity using single particle optical sensing (SPOS). This particle sizing method enables to determine the envelope volume of dispersed particles with high accuracy, provided that all particles have a spherical shape. The intraparticulate porosity ε is defined as:

$$\varepsilon = \frac{V(\text{envelope}) - V(\text{skeletal})}{V(\text{envelope})} \times 100\% = 1 - \frac{\rho(\text{envelope})}{\rho(\text{skeletal})} \times 100\% \quad (1)$$

As mentioned before the use of different intrusion media allows to gauge different fractions of the total pore volume. The different pore fractions in a material can be classified according to IUPAC into micropores, smaller than 2 nm, mesopores ranging from 2 to 50 nm and macropores, bigger than 50 nm (Burwell, 1976). The volume occupied by helium is assumed to be the total pore volume and the difference to the envelope volume is therefore the skeletal volume of the material. With nitrogen and mercury lower pore volumes are obtained corresponding to the diameter of the smallest pores into which the respective medium are able to penetrate. By comparing these values detailed information about the pore size distribution and the morphological structure can be gained. In the presented study this method was evaluated for porosity and structure analysis of poly(lactide-co-glycolide (PLGA)) microspheres prepared via an emulsion solvent extraction/evaporation process.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) 75:25 (Resomer 755 S): Mw = 64,710 Da was purchased from Boehringer Ingelheim (Ingelheim, Germany); poly(D,L-lactide-co-glycolide) 75:25 (Lactel) in granuled form was purchased from Durect Corporation (Pelham, USA); 3-{2-[4-(6-fluor-1,2-benzisoxazol-3-yl)piperidino]ethyl}-2-methyl-6,7,8,9-4-H-pyrido[1,2-a]pyrimidin-4-one was obtained by Jubilant Organosys (Mysore, India). Polyvinylalcohol 26-88 and methylene chloride analytical grade were obtained from Merck (Darmstadt, Germany), TRIS (Tris(hydroxymethyl)-aminomethan) from AppliChem (Darmstadt, Germany) and polystyrene research particles (Mean diameter 98.7 ± 1 μm) from microParticles GmbH (Berlin, Germany).

2.2. Microparticle preparation

The microparticles were prepared by an emulsification, solvent extraction/evaporation technique. 2.8 g drug substance and 3.2 g PLGA were dissolved in 40 ml of methylene chloride. The polymer solution was then emulsified in 500 ml of the extraction medium consisting of an aqueous solution of 0.5% (w/v) polyvinylalcohol and 0.1 M Tris buffer (pH 9.0). The emulsion was than feeded into a 5 L jacketed glass reactor containing 3.5 L of the aqueous phase. By stirring for 5 h the particles were hardened by solvent extraction and evaporation with an air flow of 10 l/min through the headspace of the reactor. The particles were separated by filtration and dried under vacuum in a desiccator. Different particle batches were produced by varying the temperature of the extraction mix between 10 and 35 °C.

2.3. Analytical methods

2.3.1. Single particle optical sensing (SPOS)—light obscuration

The particle size distribution was measured with an AccuSizer 780 (Sensor: LE400-05SE; Particle Sizing Systems, Santa Barbara, CA). This instrument uses the principle of light obscuration to count and size particles from 0.5 to 400 μm. The data is obtained in 512 logarithmically spaced channels with a minimum and maximum fraction width of 1–5.54 μm. Per measurement about 10 mg of microparticles were weighed exactly into a particle free vessel and dispersed in 100 ml particle free 1% Polysorbate 80 solution. For exact results it is essential, that the complete suspension is analyzed. In the case of spherical particles the total volume of a sample can be calculated from the particle size distribution. For every particle size fraction the average volume of a single particle was calculated with the sphere volume formula from the average diameter d_i of each fraction range. The total volume of all particles within a fraction was obtained by multiplication with the number n_i of particles within the respective size class. With the sum of the volumes of all 512 size fractions and the sample weight m the envelope density ρ_{env} was computed.

$$\rho_{\text{env}} = \frac{3m}{2\pi \sum_{i=1}^{512} (d_i n_i)}$$

2.3.2. Gas pycnometry

The skeletal density was measured using helium pycnometry (Ultrapycnometer 1000, Quantachrome GmbH, Odelzhausen, Germany). The samples were dried in an air flow of 3% r.h. for at least 72 h and about 500 mg particles were weighed into the medium size sample holder (volume 1.8 cm^3). The density of the sample was additionally measured with nitrogen.

2.3.3. Mercury intrusion porosimetry

In order to cover a wide pore range the mercury intrusion measurements were performed with both a high- and a low-pressure unit. As low-pressure-unit a Pascal 140 porosimeter (Thermo Fisher Scientific, I-Milano; pressure range: 0.01–400 kPa) and as high-

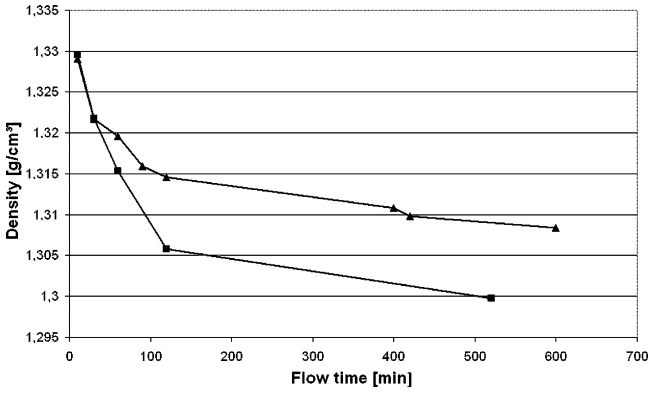

Fig. 1. Density of PLGA-microspheres as a function of drying time with dry gas flow of helium (▲) and nitrogen gas (■).

pressure-unit a Porosimeter 2000 (Carlo-Erba, I-Milano; maximum pressure: 200 MPa) were utilized.

2.3.4. Specific surface area

The specific surface area was determined by analyzing a sample of approx. 350 mg by a BET method (multi-point measurement) using a Nova 2000e surface analyzer (Quantachrome GmbH, Odelzhausen, Germany). Before the measurement the samples were degassed for 1 h at 40 °C.

2.3.5. Scanning electron microscopy

Cross sections of the microspheres were examined by scanning electron microscopy (JEOL JSM – 5310LV; JEOL Ltd., Tokyo, Japan). To study the internal structure, the particles were frozen in liquid nitrogen and cut with a razor blade. The specimens were sputtered with gold.

3. Results

A first impression of the inner structure of the microspheres can be obtained by gas pycnometry. Preliminary experiments showed, that it is essential to dry the samples completely. By purging the sample for incremental periods with the measuring gas in the sample cell of the pycnometer the moisture is slowly removed and 10 h of purging are necessary to reach constant readings (Fig. 1). In many samples, due to their microporosity, densities measured with nitrogen were lower than those measured with helium, corresponding to different molecule and atom sizes of the gases. Without pre-drying the difference between both measurements does not become apparent. Since the microspheres are not thermally stable an air flow of 3% r.h. was applied for at least 72 h to dry the samples at room temperature prior to gas pycnometry.

In order to estimate the skeletal density of the polymer matrix, pure PLGA in different morphological forms was measured as a reference material with helium and nitrogen pycnometry (Table 1). The values of the granulated and tableted PLGA and the placebo microspheres showed only slight differences between the measurements with both gases yielding a skeletal density of $1.3045 \pm 0.0027\ g/cm^3$ for helium and $1.2844 \pm 0.0077\ g/cm^3$ for nitrogen. Only the PLGA powder revealed a lower density for helium and an unexpected high value for nitrogen.

Table 1
Density of PLGA in different morphologies measured with helium and nitrogen pycnometry.

	Measuring gas	
	Helium	Nitrogen
Sample	Density [g/cm³]	Density [g/cm³]
PLGA, powder	1.2468	1.3831
PLGA, tablet[a]	1.3071	1.2884
PLGA, granules	1.3018	1.2893
PLGA, microspheres (placebo) (process temperature: 35 °C)	1.3046	1.2755
Polystyrol microparticles	1.0516	1.0536

[a] Compressed with a compactor for IR spectroscopy.

In the same way the microspheres prepared with varying process temperatures were analyzed (Table 2). The incorporated drug substance reduced the nitrogen pycnometric density more than $0.09\ g/cm^3$ below the density of the placebo microspheres. In contrast the densities measured with helium showed rather identical values differing only about $0.02\ g/cm^3$ from the placebo density. Using helium, the densities ranged between 1.2719 and $1.2853\ g/cm^3$, whereas the nitrogen pycnometric values cover a larger range from 1.1827 to $1.2784\ g/cm^3$. The differences between the helium and the nitrogen pycnometric results depend on the temperature applied in the preparation process and ranged from $0.005\ g/cm^3$ (10 °C) to $0.1\ g/cm^3$ (35 °C).

As a second parameter in Eq. (1) the envelope volume of the material is required for the calculation of the intraparticulate porosity. Taking advantage of the particles' spherical shape a method was developed to obtain the specific envelope volume and its reciprocal value, the envelope density, by optical particle counting and size fractionation of a known sample weight. In order to prove the accuracy monodisperse polystyrene microparticles with a mean diameter $98.7 \pm 1\ \mu m$ were analyzed. In case of these nonporous spherical particles the bulk density can be assumed to equal the true density of the particles. According to manufacturers data the true density of the polystyrene particles is $1.05\ g/cm^3$. By gaspycnometry a value of 1.0518 ± 0.0012 ($n = 5$) was obtained and with SPOS a density of 1.0427 ± 0.0149 ($n = 4$) was calculated, which means a deviation of less than 0.7%.

The PLGA microspheres of formulations 1–5 were found to have similar particle size distributions with median diameters (volume weighted) between 80 and 90 μm (Table 3). This corresponds well with the photomicrographs shown in Fig. 2. The calculated envelope densities varied between 0.972 and $1.053\ g/cm^3$ with higher values in case of smaller particles. The envelope density decreased with rising process temperatures, following a trend which can also be observed in nitrogen pycnometry.

The density measured by low pressure mercury intrusion porosimetry did not show a continuous temperature dependence

Table 2
Density and specific volume of PLGA microspheres prepared at different process temperatures.

Formulation	Intrusion medium	Helium		Nitrogen		Mercury (at 350 kPa)	
	Process temperature [°C]	Density [g/cm³]	Specific volume [cm³/g]	Density [g/cm³]	Specific volume [cm³/g]	Density [g/cm³]	Specific volume [cm³/g]
1	10	1.2834	0.7792	1.2784	0.7822	1.335	0.7491
2	20	1.2800	0.7813	1.2554	0.7966	1.236	0.8091
3	30	1.2719	0.7862	1.1827	0.8455	1.164	0.8591
4	32.5	1.2763	0.7835	1.1983	0.8345	1.178	0.8489
5	35	1.2853	0.7780	1.1841	0.8445	1.203	0.8313

Table 3
Volume weighted median diameter measured with SPOS and calculated envelope volume and density.

Process temperature [°C]	Median [μm]	Specific envelope volume [cm³/g]	Envelope density [g/cm³]
10	82.725	0.950	1.053
20	84.445	0.970	1.031
30	82.270	0.959	1.043
32.5	86.835	1.012	0.988
35	87.100	1.029	0.972

(Table 2). The values exceeded the envelope densities by about 0.1–0.3 g/cm³ thus indicating, that even under the conditions of low pressure Hg-porosimetry (350 kPa) mercury penetrates into the particles and consequently the values do not correctly represent the envelope volume of the microspheres.

The surface area of formulation 5 was determined by nitrogen adsorption. Although SEM photomicrographs revealed a highly porous structure, the value for the surface area of 0.41 m²/g was unexpectedly low.

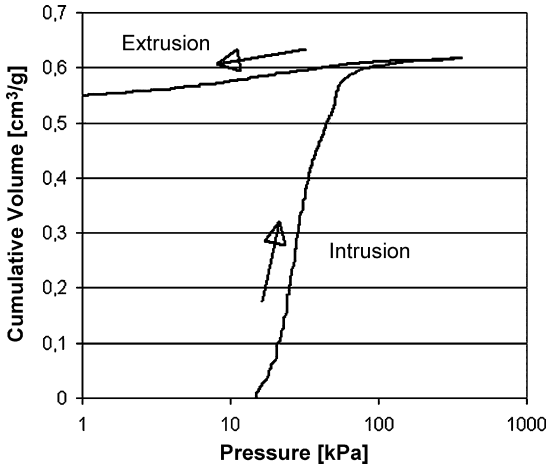

Fig. 3. Intrusion–extrusion curve of monodisperse polystyrene microspheres (⌀ 98.7 ± 1 μm).

4. Discussion

4.1. Interparticulate volume

The main focus of this investigation was the intraparticulate volume. However, a bulk material like microspheres contain also another type of voids: the interparticulate space. Fig. 3 shows the mercury intrusion–extrusion curve of monodisperse polystyrene spheres with a diameter of 98.7 μm. The intruded mercury fills the interparticulate space until its volume reaches a plateau value of 0.603 cm³/g at 100 kPa.

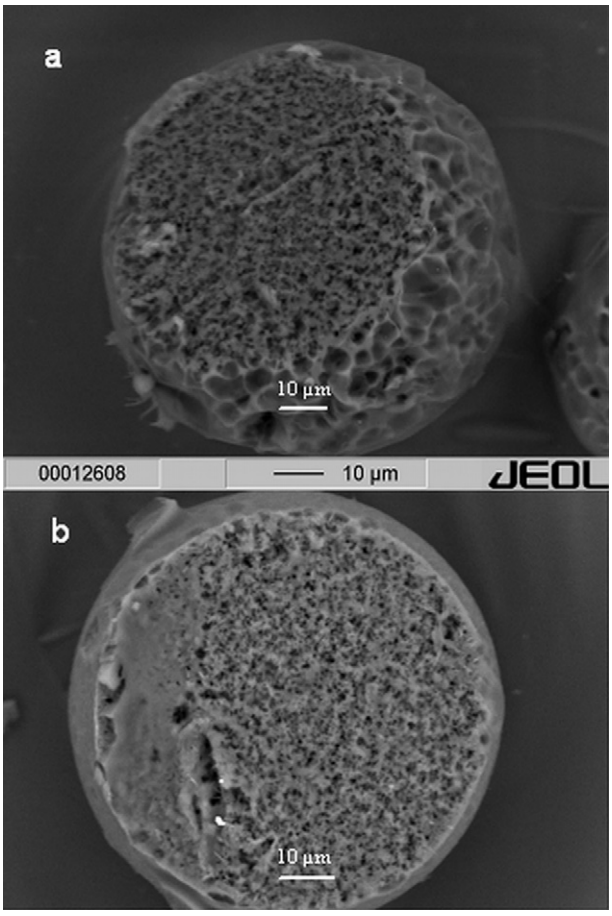

Fig. 2. SEM of PLGA microspheres prepared at 10 °C (top) and 35 °C (bottom).

This pressure corresponds to a pore diameter of 17 μm which is exactly 1/6 of the particle diameter, the value expected on basis of a consideration of Tonellier (Tonnellier, 2008). He reports that the diameter of voids between monodisperse spheres is about 1/6 of the particle diameter. It can be concluded that it is even smaller in case of a heterogenous size distribution where small particles fill the voids between larger ones. The small slope of the plateau can be attributed to a pressure-induced deformation of the polymer structure. It leads to an intrusion volume of 0.618 cm³/g at 350 kPa, the pressure which was found necessary to fill also the interparticulate voids between the smaller particles of the PLGA formulations. The sum of this intrusion volume and the specific volume of the polystyrene spheres measured at the same pressure of 350 kPa (0.929 cm³/g) is the bulk volume of the particle bed (1.547 cm³/g). Its packing density, 61.99%, is obtained as the ratio of the envelope volume, determined by the SPOS method (0.959 cm³/g), and the bulk volume (0.959 cm³/g/1.547 cm³/g = 61.99%). In case of non-porous particles about the same value can also be obtained from mercury porosimetry data only as the ratio of the specific volume and the bulk volume (0.929 cm³/g/1.547 cm³/g = 60.05%). Both results are close to the maximum random packing density of monodisperse spheres which was calculated as 64.35% (Farr and Groot, 2009). As discussed before, in case of porous PLGA microspheres the mercury intrusion volume covers the interparticulate voids in addition to a certain part of the intraparticulate pores (>3.9 μm at 350 kPa). The specific volume measured at the same mercury pressure is the skeletal volume plus the remaining pore volume (<3.9 μm). Table 4 shows the mercury intrusion volume, the specific volume (both determined at 350 kPa), and the bulk volume of formulations 1–5. The difference to 100% is the share of the interparticulate voids in the total bulk volume. The particles of all formulations are about the same size and log-normal distributed with a standard deviation of 0.26–0.36.

According to Farr the maximum packing fraction of a log-normal sphere distribution depends on its standard deviation σ_ζ and amounts to 67–68% for the mentioned range of σ_ζ. Because of the intraparticulate porosity it is not surprising to find values smaller than theoretically expected (58–61%) if the packing density is calculated as the ratio of the specific volume and the bulk volume, both determined with mercury at 350 kPa. If the calculation is done with the envelope volume measured by SPOS, however, packing densities about 5% higher than predicted are obtained (72–74%). This can be explained by considering that the bulk volumes included

Table 4
Intrusion, specific and bulk volume for formulations 1–5 and the resulting packing density.

Formulation	Intrusion volume [cm³/g] at 350 kPa	Specific volume [cm³/g] at 350 kPa	Bulk volume [cm³/g]	Packing density[a] [%]
1	0.5292	0.7491	1.2782	74.32
2	0.5159	0.8091	1.3249	73.21
3	–	0.8591	–	–
4	0.5478	0.8489	1.3968	72.59
5	0.6017	0.8313	1.4327	72.24

[a] Packing density = specific envelope volume (from Table 3)/bulk volume.

in the calculation are determined with mercury under a pressure of 350 kPa. Due to the compression of the particle structure these values are smaller than they would be in an unpressurized state leading to an overestimation of the packing density.

4.2. Intraparticulate volume

According to their atomic or molecule diameters, the chosen intrusion media can penetrate to different degrees into the microspheres. They allow to determine the intraparticulate volume, but render different results. There are several options to define the spatial dimensions of an atom or molecule. The kinetic diameter provides the most appropriate information for the estimation of the accessible pore size. This diameter – 0.36 nm for nitrogen and 0.26 nm for helium (Breck, 1974) – represents the diameter of the smallest pores into which the molecules or atoms can just penetrate. With these measuring gases the lower range of the microporosity can be determined. In case of mercury the intrusion capability depends on the applied pressure. Under the assumption of a cylindrical shape the minimum diameter d_p of mercury-accessible pores, can be calculated from the pressure p using the Washburn equation:

$$d_p = -\frac{4\gamma \cos\theta}{p} \quad (2)$$

with a contact angle θ of 135° between mercury and PLGA and a surface tension γ of 485 mN/m (Mikos et al., 1994). At 350 kPa, the pressure applied during the low pressure measurement, pores with a minimum diameter of 3.9 μm are filled and sized and with a pressure of 200 MPa pores down to 6.9 nm are detected. This implies that the high pressure mode of mercury intrusion porosimetry is only suitable to determine pores in the meso- and macroporous range, whereas micropores can only be measured by gas pycnometry.

As reference the helium and nitrogen pycnometric densities were determined for the pure PLGA in different morphological forms, which showed consistent values. Only the PLGA powder shows different values (ρ(He):1.2468 g/cm³ and $\rho(N_2)$:1.3831 g/cm³) which cannot be adequately explained.

Highest skeletal densities were found with pure PLGA granules and with the compressed polymer. The granules are transparent, which is an indication for a virtually pore-free, compact structure. A highly dense structure was also measured with particles prepared at 10 °C. The He- and N_2-pycnometric densities are only slightly lower than the values obtained with PLGA granules (He: $\Delta\rho$ = 1.41%, N_2: $\Delta\rho$ = 1.27%) or compressed PLGA (He: $\Delta\rho$ = 1.81%, N_2: $\Delta\rho$ = 1.28%). Despite the similar gaspycnometric densities, formulation 1 clearly differs from PLGA granules and tablets in its morphology and internal microstructure. This demonstrates that the skeletal density alone does not allow, to distinguish between a compact body and a porous material.

Different information on the intraparticulate volume is provided by the specific envelope volume, which is the reciprocal of the envelope density. Often it is assumed, that the specific envelope volume can be determined by low-pressure mercury intrusion porosimetry. Comparison of the specific volumes obtained by this method

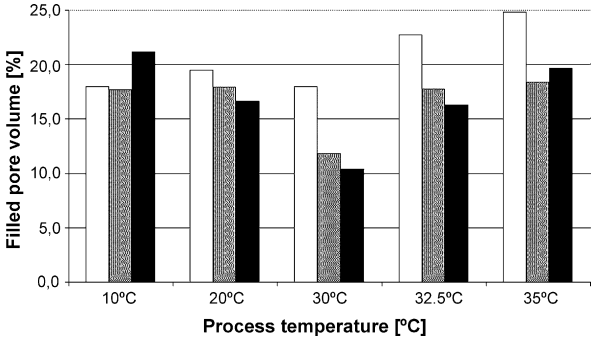

Fig. 4. Pore volumes filled by helium (□), nitrogen (▨) and mercury (■).

(0.7491–0.8591 cm³/g) (Table 2) with the envelope volumes calculated from SPOS (0.950–1.029 cm³/g) (Table 3) shows that, even under low pressure, mercury penetrates into the microspheres to a substantial degree. This is in accordance with Tonnellier (Tonnellier, 2008), who found that mercury intrusion porosimetry is not an appropriate method to distinguish between intra- and interparticulate porosity. In order to measure the envelope volume of the spheres the intrusion medium must completely fill the interparticulate voids without infiltration of the porous particles. Because all the tested samples are particle fractions between 30 and 150 μm the smallest interspaces can be rated to about 5 μm (=30/6 μm) according to the abovementioned consideration. According to the Washburn equation a pressure of about 350 kPa is necessary to guarantee that all these interparticulate pores (down to a theoretical diameter of 3.9 μm) are filled. As a consequence, however, mercury accesses also a certain fraction of intraparticulate pores which are in a similar size range (Fig. 2). Hence at least in case of samples with a broad size distribution, where the smallest interparticulate voids are similar in size to the largest intraparticulate pores, mercury intrusion is not a suitable method for determination of the particles' envelope volume. Provided that the sample consists of spherical particles only, the optical method we have developed allows a precise determination of this parameter.

4.3. Porosity profiles

The principle of the described method for studying the pore size distribution of microspheres is the combined application of three different intrusion media and an optical particle sizing method. The envelope volume is confined by a convex hull around the outer dimensions of each particle, whereas the specific volume, the reciprocal value of the helium pycnometric density, represents the volume of the mere polymer matrix. Thus the difference between both is the volume of voids within the material. Due to its smaller intrusion capacity a different pore volume is calculated when nitrogen is used instead of helium. Yet another value is obtained by mercury intrusion porosimetry. By combining these data porosity profiles can be obtained which provide information on the absolute pore volume and the pore size distribution. Fig. 4 depicts the

different types of the pore volume as percentages of the total particle volume, i.e. the envelope volume. The highest pore volume can be detected with helium, as its atoms have the smallest kinetic diameter of all applied intrusion media. An exception is formulation 1, which shows a pore volume filled by mercury of 21.2%, but a pore volume filled by helium of only 18%. This can also be interpreted as a result of a substantial collapse of the more fragile internal pore structure under the applied pressure during the mercury porosimetry measurement.

The volume of these helium-accessible pores shows an increase from 18% for the sample prepared at 10 °C (formulation 1) to 24.8% for the microspheres prepared at 35 °C (formulation 5). Neither the pycnometric data nor the mercury porosimetric measurements reflect this trend because it is almost exclusively caused by the change of the envelope volume. This emphasizes once more the importance of this parameter. In case of formulations 1 and 2 (preparation temperature 10 °C and 20 °C, respectively) there is only little difference between the helium and nitrogen pycnometric data. This is an indication of a coarse pore structure with large pores, embedded in a tightly packed polymer matrix. These particles reveal an open macroporous structure which is highly accessible to all intrusion media (Fig. 2a) and microporosity is very low. Formulation 2 reveals a more graduated pore size distribution with a small fraction of microporosity but more than 85% of the total pore volume consist of voids larger than 3.9 μm.

The presence of a small fraction of micropores in formulations 1 and 2 can be attributed to an incomplete solvent extraction at low temperatures (3% methylene chloride remaining after 6 h extraction). As drying took place at room temperature and thus above the glass transition temperature (21 °C), the PLGA matrix could densify since the polymer chains were still flexible resulting in a loss of microporosity.

During extraction at higher temperatures a densified shell is formed around the particles as can be seen in Fig. 2b (Jeyanthi et al., 1996; Allison, 2008). At 30 °C the superficial solidification has to be regarded as a still relatively slow process which passes an extended transitional stage with a fragile polymer skin surrounding the solidifying droplets. Frequent disruptions of this skin resulting in a release of solvent portions and shrinkage of the particles could explain the porosity profile of formulation 3. The total porosity is similar to the one of formulations 1 and 2, but a substantial fraction of micropores is detected, whereas the macro- and mesoporosity is strongly reduced.

With an increase in process temperature, the structure changes and the differences between helium and nitrogen measurements become more distinctive. Between 30 and 35 °C the total porosity increases from 18% to 24.8% and a fraction of micropores occurs at 30 °C which is only accessible to helium but not to nitrogen. Expressed as a proportion of the total pore volume this subfraction of micropores smaller than 0.36 nm increases abruptly from 2–8% up to 22–34% as the preparation temperature reaches 30 °C. This suggests, that the structure becomes finely ramified, so that only small helium atoms can diffuse into the end sections of the pores, whereas they are inaccessible to nitrogen. These "submicropores" are the main reason for the increase of total porosity at elevated extraction temperatures. At a process temperature above 30 °C the polymer matrix is well hydrated and the concentration of residual solvent is very low at the end of the extraction process (<0.7%). The glass transition temperature is therefore above room temperature (>31 °C) and the polymer is dried in a rigid state. Under these conditions water acts as a porogen leaving sub-nanometer voids in the places from which water molecules are removed.

In case of all formulations tested nitrogen and mercury fill almost the same fraction of pores. Although nitrogen is able to penetrate into pores which are about 10,000 times smaller than those accessible to mercury at 350 kPa the pore volume measured with nitrogen is not more than 1.4% higher than the volume determined with mercury. Helium, however, which atoms are only a little smaller than N_2 molecules with respect to their kinetic diameters, is able to reach an additional pore volume of up to 6.4% compared to nitrogen. This indicates the presence of two separate pore populations, one smaller than 0.36 nm and another larger than 3.9 μm, with not more than 6% of the total porosity lying in between. This is the reason for a rather low BET surface area of only 0.41 m^2/g. From the particle size distribution the envelope surface area of the microspheres was calculated as 0.07 m^2/g, which amounts already to 17% of the total specific surface area. Although the microspheres exhibit pores to a substantial degree (Fig. 2) their internal surface area is only about 0.34 m^2/g. This can be explained by the fact, that on the one hand macropores larger than 50 μm contribute only little to the surface area and on the other hand the majority of the micropores is smaller than the kinetic diameter of nitrogen and is therefore not accessible to the measuring gas used for the BET measurements.

The appearance of two separate intraparticulate pore populations suggests their formation by different mechanisms. The larger voids are obviously created by shrinkage and rupture of the drying polymer or are a result of larger channels and pockets in the material, which where initially filled with sequestered water (Nihant et al., 1994; Li et al., 1995). The micropores, by contrast, are the remaining vacancies which are formed when water molecules evaporate from the hydrated polymer (Yang et al., 2000).

5. Conclusions

As the porosity represents a central feature of microspheres for pharmaceutical use as well as for other applications it is a key to understanding the mechanisms of formation and behaviour of such complex structured materials. So far the porosity is often estimated by SEM micrographs, but this method provides only information about the macroporous structures inside the microspheres. Mercury intrusion porosimetry is an established method to determine the specific pore volume and the pore size distribution of solid materials but (i) does not provide any information on the porosity as defined by ASTM, (ii) is not able to distinguish between inter- and intraparticulate porosity, and (iii) is often biased by compression induced structural changes of the specimen. Because of these limitations further techniques are needed to supplement these methods. Our novel combined method is able to determine the porosity according to the ASTM definition (pore volume per total volume) and provides "fingerprints" of the pore size distribution covering a larger size range than any single method. At least three different pore size fractions can be distinguished including micropores smaller than 0.36 nm, in which only helium can diffuse, pores ranging from 0.36 nm to 3.9 μm in which nitrogen, but not mercury can penetrate, and macropores larger than 3.9 μm which are accessible to all intrusion media. With this comprehensive information it is possible to uncover structural properties even in the submicroscopic scale thus gaining deeper insight into the application-specific functionalities of microparticles.

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