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Influence of gas density and pressure on microparticles produced with the ASES process

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Abstract

The aerosol solvent extraction system (ASES) uses the extraction properties of supercritical gases to produce microparticles. The extraction properties of the gas depend on pressure, temperature and the adjusted density. As part of a factorial design systematic investigations are carried out on the influence of pressure (in the range of 90–200 bar) and density of the supercritical carbon dioxide (in the range of 0.5–0.8 g/ml) on yield, particle morphology, particle size and release properties of the particles. The results show that the properties of the produced microparticles are hardly influenced by the different production conditions.

Key words: Supercritical gas; Supercritical fluid extraction; Gas density; Biodegradable polymer; Microparticle

1. Introduction

The aerosol solvent extraction system (ASES) uses the extraction properties of supercritical gases to produce microparticles (Müller and Fischer, 1991). Drug and polymer are dissolved or dispersed in an organic solvent which is sprayed into the supercritical gas phase (in this case carbon dioxide). The organic solvent is soluble in the supercritical gas and is extracted leading to the formation of solid microparticles. The extraction properties or the solvent power of supercritical fluids depend on pressure, temperature (Hubert and Vitzhum, 1980) and the adjusted density. The density of the gas is a function of pressure and temperature and can be varied over a wide range. Different densities lead to different solubilities of substances which are dissolved in a supercritical gas phase. Wong and Johnston (1986) demonstrated that the solubility of different solid sterols in supercritical carbon dioxide increased with the density (obtained with increasing pressure). Stahl et al. (1980) showed that the solubility of seven different substances (glycine, frangulin, emodin, *p*-hydroxybenzoic acid, 1,8-dihydroxyanthraquinone, salicylic acid and benzoic acid) depends on the adjusted density of the supercritical carbon dioxide. Stahl adjusted the density by varying the pressure at a constant temperature of 40°C. Higher densities lead to greater solubility in the supercritical fluid. These results lead to the assumption that the gas density during the ASES process can have an influence on the microparticle properties.

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2. Materials and methods

2.1. Microparticle production

The biodegradable polymer used was a poly(L-lactide) (Resomer L-206, Mol. Wt 102000) and the model drug was hyoscine butylbromide, b.p. 88°C. Drug and polymer were dissolved in methylene chloride/methanol (85 : 15) which was sprayed into the supercritical gas phase (in this case carbon dioxide). The organic solvent is soluble in the supercritical gas and is extracted, leading to the formation of microparticles. The production of microparticles was carried out with a static carbon dioxide phase as described earlier (Bleich et al., 1993). As part of a factorial design, systematic investigations were carried out over the pressure range of 90–200 bar and density range of 0.5–0.8 g/ml.

2.2. Calculation of the gas density

The density data available in the literature were not sufficient to set up an experimental plan. For this reason the densities were calculated using the general gas equation. For real gases the equation is extended with the compressibility factor Z (Eq. 1):

$$pV = ZnRT \tag{1}$$

$$\rho^* = \frac{n}{V} = \frac{p}{RT} \cdot \frac{1}{Z} \tag{2}$$

where ρ^* is the molar density (mol/m^3), n the number of moles (mol), V the volume (m^3), p the pressure (Pa), R the general gas constant (J mol^{-1} K^{-1}), T the temperature (K) and Z the compressibility factor.

For an ideal gas $Z = 1$ and for non-ideal gases $Z \neq 1$. The deviation from the ideal gases depends on temperature and pressure, $Z = f(p,T)$. The compressibility factor Z can be calculated using Eq. 3 (Lee and Kesler, 1975):

$$Z = Z^{(0)} + \omega Z^{(1)} \tag{3}$$

$Z^{(0)}$ and $Z^{(1)}$ are assumed functions of the reduced pressure ($p_r = p/p_{critical}$) and the reduced temperature ($T_r = T/T_{critical}$). To calculate Z for a given temperature and pressure, first the values for T_r and p_r are calculated using the

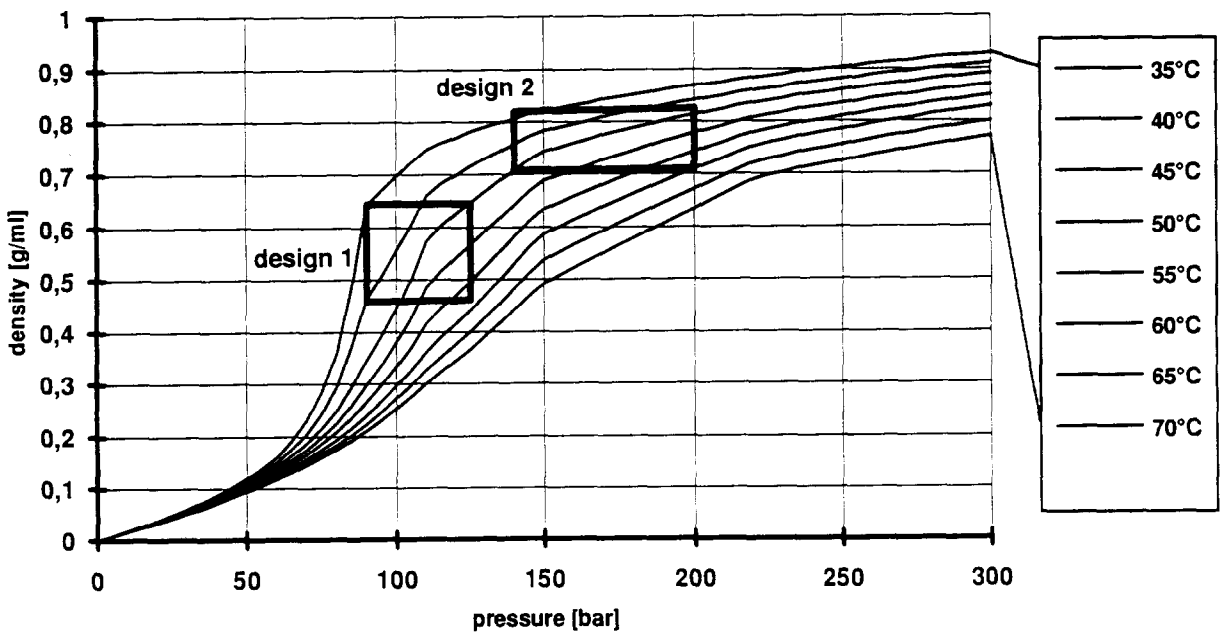

Fig. 1. Dependence of the density of carbon dioxide on pressure and temperature.

Table 1
Pressure and density of the experiments: upper, design I; lower, design II

CO_2 density (g/ml)	Pressure (bar)	Temperature (°C)
0.46	90	40
0.46	125	58
0.65	90	35
0.65	125	45
0.71	140	45
0.71	200	60
0.82	140	33
0.82	200	43

critical properties of carbon dioxide ($T_{critical}$ = 304.2 K and $p_{critical}$ = 73.8 bar). Then the values for $Z^{(0)}$ and $Z^{(1)}$ can be taken from tables reported by Lee and Kesler (1975) who also described the calculation of $Z^{(0)}$ and $Z^{(1)}$. The acentric factor ω is a constant depending on the geometric molecular structure. For carbon dioxide $\omega = 0.225$ (VDI-Waermeatlas, 1988). The calculated densities lead to the isotherms in the pressure-density diagram (Fig. 1).

2.3. Experimental design

In this experiment upper and lower values for temperature limits had to be taken in account. The lower temperature limit (31°C) was given by the critical temperature of carbon dioxide. On the other hand, an upper limit of 60°C is given by the technical equipment. To cover a wide range of density with respect to the temperature limits it was necessary to work with two designs. In both experiments one corner of the factor space exceeded the glass transition temperature of the polymer. The pressure and the density of the experimental runs according to the designs are listed in Table 1 and the positions of the designs are shown in Fig. 1.

2.4. Yield

The yield was determined by weighing the microparticles and calculating the percentual yield in relation to the used amount of drug and polymer in the experiment. For the evaluation of differences between the experimental runs the amount of microparticles in the collecting container was determined separately and the percentage in relation to the total yield was calculated.

2.5. Particle morphology (scanning electron microscopy; SEM)

The particles were fixed using a conductive tape (Plano, Marburg, Germany) and were coated with gold for 60 s under an argon atmosphere (Mini Coater Commonwealth Scientific, Alexandria, VA, U.S.A.). The shape and surface characteristics of the produced microspheres were examined with a Stereoscan S4-10 (Cambridge Instruments, Cambridge, U.K.).

2.6. Particle size measurement

The particles were suspended in a 0.01% (m/v) solution of Pluronic F68, stirred for 30 s with a laboratory stirrer (Model REAX 1DR, Heidolph, Kelheim, Germany). The particle size distribution was measured with a laser diffractometer (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). In a second sample preparation method the particles were suspended in a 0.01% (m/v) solution of Pluronic F68, stirred for 30 s and sonicated (80 W, 35 kHz) prior to particle size measurement. The optimal time for the sonication was investigated with two microparticle batches over a period of 180 s. The deagglomeration process was finished after 90 s (Fig. 2). The volume size

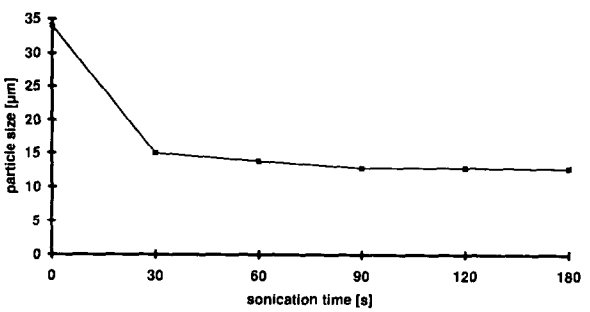

Fig. 2. Determination of the optimal sonication treatment time.

Table 2
Results of the particle size measurement

Pressure (bar)	Density (g/ml)	$X10$ (μm)	$X50$ (μm)	$X90$ (μm)	$X90-X10$ (μm)	$(X90-X10)/X50$
90	0.46	9.84	40.31	82.6	72.76	1.81
125	0.46	29.47	91.22	148.09	118.63	1.30
90	0.65	7.88	31.60	64.39	56.51	1.79
125	0.65	13.45	43.68	84.13	70.68	1.62
140	0.71	8.26	28.61	57.53	49.27	1.71
200	0.71	21.02	64.37	138.12	117.1	1.82
140	0.82	9.46	37.89	107.31	69.17	1.83
200	0.82	9.08	30.54	62.95	53.87	1.76

distribution was calculated with a computer program (Sympatec). The 10, 50 and 90% quantiles ($X10$, $X50$, $X90$) of the size distribution are listed in Table 2. $X90$ minus $X10$ was calculated to express the distribution width and ($X90$ minus $X10$) divided by $X50$ was used to describe the relation between distribution width and average size.

2.7. Drug content

Sample preparation: 25 mg microparticles were suspended in 25 ml of a mixture of acetonitrile and methanol (40:10 v/v). This suspension was sonicated for 1 h and 0.001 M acetic acid was added up to 50 ml.

The drug content was determined using an HPLC method. The HPLC system consisted of a pump (Model 300B, Gynkotech, München, Germany) connected to a Hypersil SAS column (250 × 4.6 mm) (Techlab, Erkerode, Germany), a guard column (40 × 4.6 mm) (Techlab) and an autosampler (Model 465, Kontron, München, Germany), equipped with a 200 μl loop. The mobile phase (pH 7.4) consisted of acetonitrile/0.01 M KH_2PO_4 (60:40 v/v). The flow rate was 2 ml/min. Detection was performed by means of a UV detector (Model Uvikon 735 LC, Kontron) operated at a wavelength of 220 nm. The data were processed with the aid of a computer system using the DS 450-MT software (Kontron).

2.8. Drug release

The release rate was determined by means of a USP paddle apparatus II (Model PTW S, Pharmatechnik, Hainburg, Germany) at a rotation speed of 100 rpm. The pH optimum for the stability of hyoscine butylbromide is in the range of pH 3–4.6. Higher pH values and rising temperatures result in hydrolysis of the drug. Thus, the dissolution test was carried out in Sörensen phosphate buffer pH 5.0 (Richterich and Colombo, 1978) at a temperature of 25.0°C (±0.2°C). Isotonicity was adjusted by adding sodium chloride. The drug release from 0.15 g microparticles was tested in a volume of 350 ml dissolution medium. Samples were withdrawn periodically over 30 h and buffer was substituted for the volume removed. The concentration of dissolved drug was determined using the HPLC method mentioned above.

3. Results and discussion

The mean value of the microparticle yield is 81% (s = 1.69%). The amounts in the collecting container (in percentage of the total yield) are shown in Fig. 3. The low yield in two experiments (125 bar, 0.46 g/ml [58°C], design I and 200 bar, 0.71 g/ml [60°C], design II) can be explained by the adjusted temperature. This was above the glass transition temperature and the particles adhered to the column wall. The low yields in the first design (only one experiment had a yield between 80 and 90%) cannot be explained. Electrostatic phenomena may be a reason. Electrostatic charge can occur with micronized polymers and this is a common problem when they are used in the laboratory (Nordmann, 1990). In the second design the yield in the collecting container is in the range between 80 and 90% apart from the exception mentioned above. All resultant powders have good flow properties.

The SEM studies (Fig. 4) show that under all experimental conditions spherical particles are obtained which partially form agglomerates (Fig. 5). Particles which were produced above the glass transition temperature have a distinctly greater

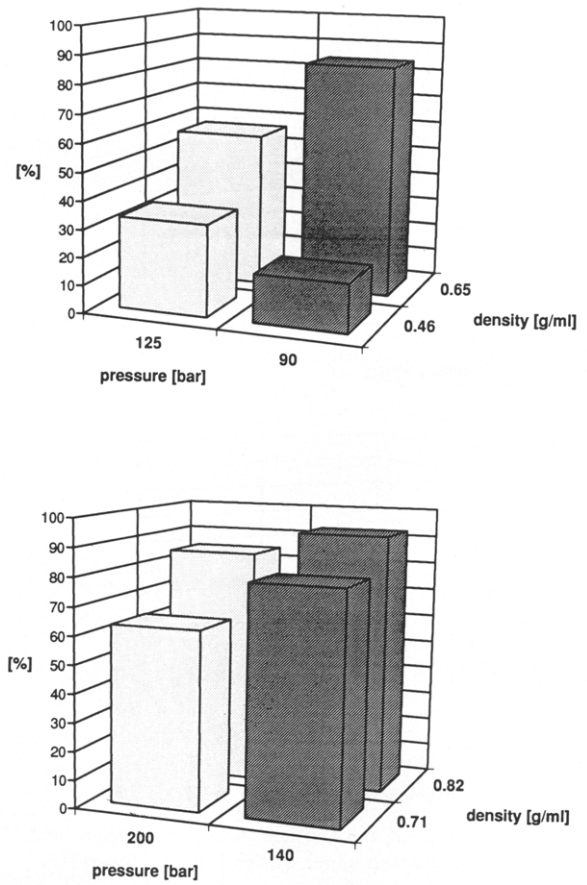

Fig. 3. Yield in the collecting vessel (percentage of the total yield): upper, design I; lower, design II.

tendency to form agglomerations (Fig. 6). It was observed that the particles stick together leading to a tight connection. The properties of these microparticles are different from those which were produced at lower temperatures. It can be seen macroscopically that the powder is of a coarser texture and that there are larger agglomerations.

All experimental runs lead to microparticles with a drug content between 14.7 and 15.3%. This is equivalent to 98.4–101.7% of the expected value. This means that the there are no problems associated with drug or polymer extraction within the components used. The examination was necessary because the solubility of substances in supercritical carbon dioxide depends on their polarity (Stahl and Glatz, 1984). Polymers with low molecular weight can be dissolved in supercritical carbon dioxide (Lele and Shine, 1990) and supercritical fluid extraction is a common process for the purification of polymers (Braun et al., 1985).

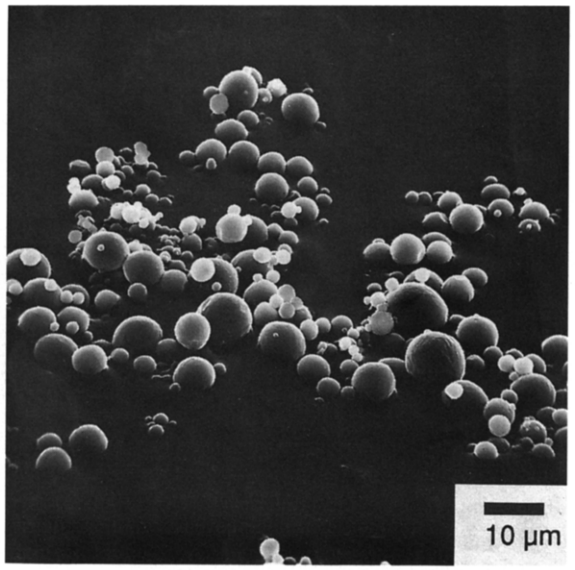

Fig. 4. Particle photo: separated microparticles.

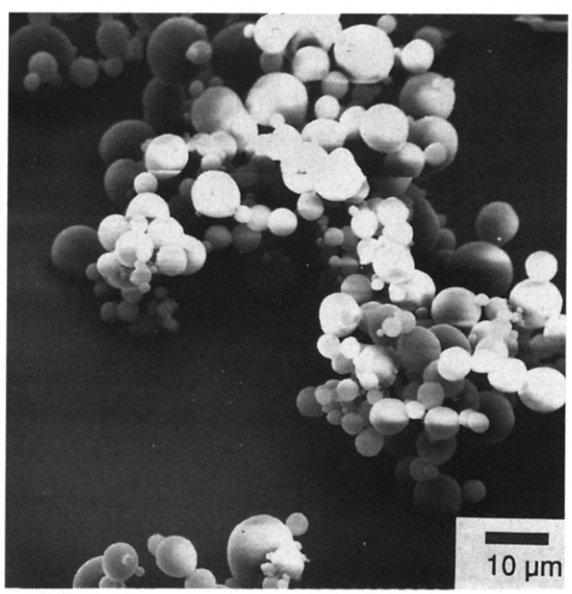

Fig. 5. Particle photo: agglomerated microparticles.

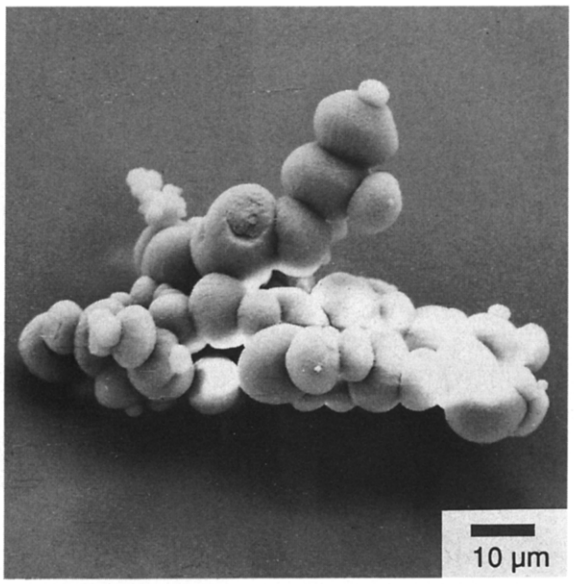

Fig. 6. Particle photo: microparticles agglomerated above the glass transition temperature.

The quantiles $X10\%$, $X50\%$ and $X90\%$ of the particle size measurement are listed in Table 2 and the results are shown in Fig. 7 and 8. The particle size without sonication is greater by a factor of between 2 and 3 than with ultrasonic treatment. In the SEM study it can be seen that the microparticles produced form agglomerates of microparticles with a size smaller than 20 μm (Fig. 5). The deagglomeration is not possible via a normal agitation process (30 s stirring in 0.01% Pluronic F-68). After sonication the $X50$ values

Table 3
Results of the particle size measurement after sonication treatment

Pressure (bar)	Density (g/ml)	$X10$ (μm)	$X50$ (μm)	$X90$ (μm)	$X90-X10$ (μm)	$(X90-X10)/X50$
90	0.46	2.00	12.31	23.64	21.64	1.76
125	0.46	9.29	28.29	49.09	39.81	1.40
90	0.65	1.87	11.12	21.15	19.29	1.73
125	0.65	4.86	19.71	37.50	32.64	1.66
140	0.71	2.26	13.69	25.86	23.61	1.72
200	0.71	15.84	41.80	70.30	55.47	1.33
140	0.82	2.39	13.62	25.84	23.45	1.72
200	0.82	2.45	14.09	26.95	24.50	1.74

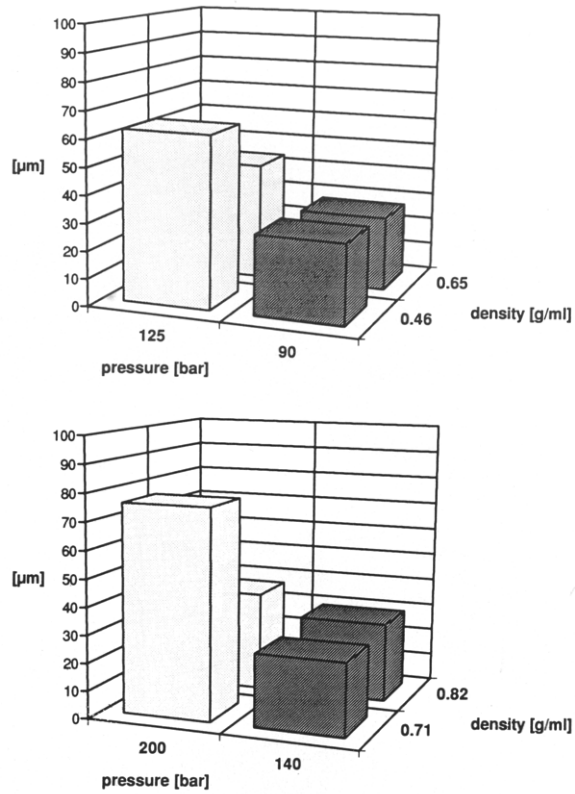

Fig. 7. Results of the particle size measurement without supersonic treatment: upper, design I; lower, design II.

are less than 20 μm and the distribution width is in the range of 20–30 μm except the two experiments at 125 bar, 0.46 g/ml [58°C], design I and at 200 bar/0.71 g/ml [60°C], design II. The particles produced at higher temperatures lead to compact agglomerates by bridging (larger particle size). Agglomerates formed in these experiments lead to distribution widths above 100 μm. There is no correlation possible between production parameters, particle size and distribution width, respectively (Fig. 7). This indicates that different carbon dioxide densities have no influence on the microparticle size in the ASES process. An explanation for the lack of influence of gas density and gas pressure on microparticle properties could be that the formation of microparticles is a precipitation process. The solvent is soluble in the supercritical carbon dioxide phase while polymer and drug are insoluble in the resulting mixture of

supercritical gas and organic solvent leading to the formation of microparticles. A similar sort of precipitation process was developed by Leelarasamee et al. (1986, 1988). The method involves continuos injection of a drug-polymer solution with a syringe infusion pump into flowing mineral oil where microcapsules are formed as the solvent of the drug and and the polymer is partitioned into the mineral oil. Another precipitation process for the preparation of liposomes, however, in liquids has been described by Batzri and Korn (1973). They injected an ethanolic solution of phospholipid into an aqueous (salt) solution. This procedure results in a homogeneous preparation of vesicles.

All release profiles obtained (Fig. 9) show a marked burst effect. During the first 2 h more than 50% of the drug is released. Subsequently, a plateau is reached. This indicates that there is a larger amount of the drug at the surface. The remaining drug is released very slowly from the particle matrix. In further studies the incorporation of the drug into the polymer matrix may be adjusted by using a suspension of the drug in a polymer solution employing this microparticle production technique. To overcome the slow release of the drug, other polymers such as polylactic-glycolic acid must be tested. The water uptake and swelling characteristics of these polymers are stronger (Kissel et al., 1985) and here drug dissolution may be better.

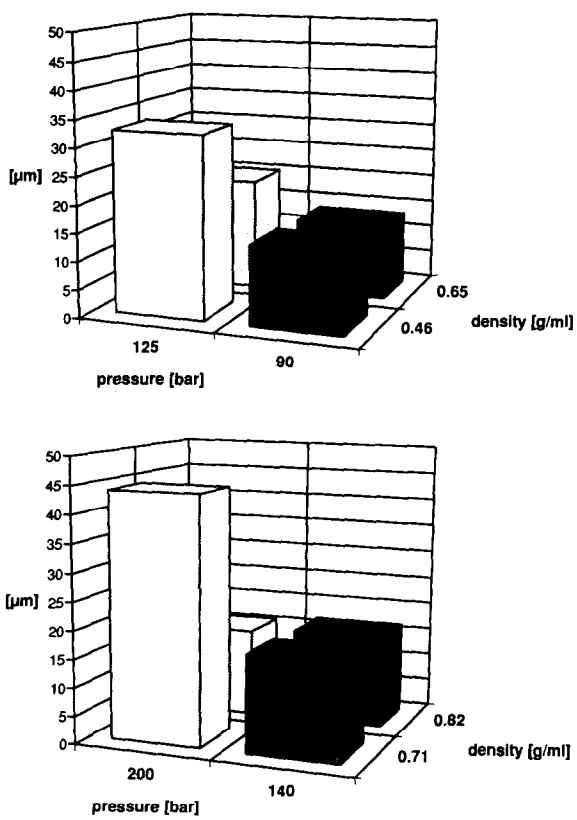

Fig. 8. Results of the particle size measurement after supersonic treatment: upper, design I; lower, design II.

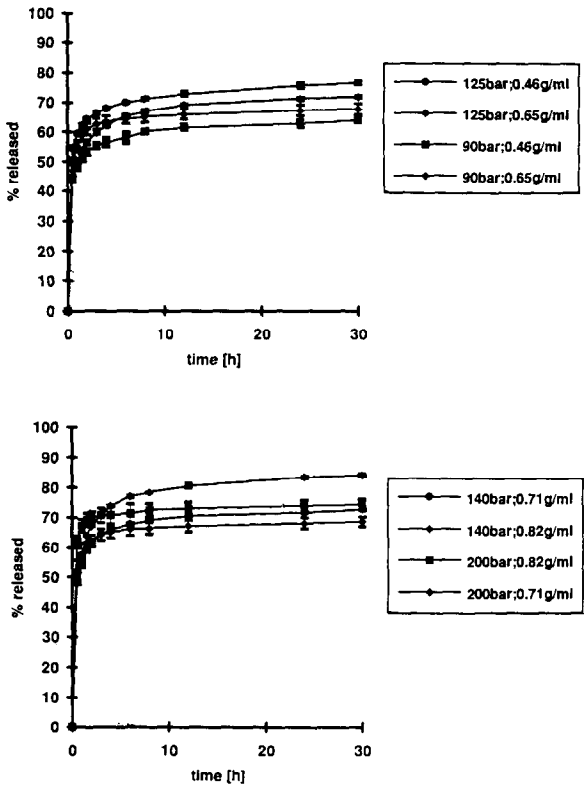

Fig. 9. Drug release: upper, design I; lower, design II.

4. Conclusions

The present results indicate that different carbon dioxide densities and gas pressures had no effect on the investigated properties of the mi-

croparticles. Greater particle sizes in two experiments were due to the high temperatures which were needed to achieve the densities at the given pressures.

5. Acknowledgement

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