Effect of Preparation Temperature in Solvent Evaporation Process on Eudragit RS Microsphere Properties

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Eudragit RS 100 microspheres containing ketoprofen as a model drug were prepared by the solvent evaporation method using an acetone/liquid paraffin solvent system. The influence of various preparation temperatures: 10, 25, 35, and 40 °C, on particle size and morphology, drug content and release kinetics, and drug crystal state was evaluated. With increasing temperature, microsphere average size was found to increase and particle size distribution to widen significantly. At 10 °C particles of irregular shape are formed, whereas higher temperatures gradually improve the sphericity of microspheres. As can be seen from SEM photographs, particle surface roughness decreases as preparation temperature increases. It was found that temperature had no effect either on ketoprofen microencapsulation efficiency or on its crystal state, but it does influence emulsion-stabilizer incorporation. Ketoprofen forms solid solution in Eudragit matrix and maintains amorphous state for significant period of time. Drug release rates from microspheres correlated with microspheres' surface roughness and to a lesser extent with particle size.

Key words microsphere; solvent evaporation; preparation temperature; high sensitivity differential scanning calorimetry (HSDSC)

Microencapsulation by the solvent evaporation method is a complex process, which can be influenced by many process parameters, *e.g.* solvent evaporation rate,[1)] temperature,[2—5)] solubility of polymer, drug and excipients in both emulsion phases,[6,7)] dispersion stirring rate,[7—9)] viscosity, solubility, volume and volume ratio between the inner and outer phases,[10—12)] the quantity of polymer and drug,[1,6)] and the physico-chemical properties and concentration of the stabilizator.[11,13)] Some authors have previously studied the effects of preparation temperature on microsphere formation and characteristics: mean microsphere diameter and size distribution width,[2,5)] particles morphology,[2)] porosity,[5)] and drug loading.[5)]

One can find only one report on the effect of temperature on microspheres containing Eudragit. We have previously reported that temperature (40, 50, 60 °C) had an insignificant effect on mean diameter or drug encapsulation yield using Eudragit E as a matrix polymer.[3)] However, these results differ from the findings of the above mentioned authors.[2,5)] Thus, the objective of this study was to investigate the effect of preparation temperature on Eudragit RS microsphere properties in a different temperature range. Lower temperatures were examined, where more pronounced influences were expected. Average particle size and microsphere morphology, drug content and release kinetics, and drug crystal state in microspheres were evaluated.

Experimental

Materials Eudragit RS were kindly provided by Röhm GmbH, Darmstadt, Germany, magnesium stearate and ketoprofen by Lek Pharmaceuticals d.d., Slovenia. All other substances used were of analytical grade.

Microsphere Preparation Microspheres were prepared using emulsification and a solvent evaporation technique in an acetone/liquid paraffin solvent system. 1.25 g of Eudragit RS and 0.75 g of ketoprofen were dissolved in 5 ml of acetone. 0.35 g of magnesium stearate was separately dispersed in 3 ml of acetone and added to the mentioned solution. The dispersion of magnesium stearate, Eudragit RS and ketoprofen in acetone was emulsified into 80 ml of liquid paraffin with fixed temperatures (10, 25, 35, 40 °C). The system was stirred at 250 rpm at the above-mentioned constant temperatures for 1 h. The microspheres were filtered, washed with *n*-hexane, and dried overnight at room temperature under reduced pressure. Microspheres were prepared in triplicates at each of the defined temperatures. All the experiments for microsphere characterization were performed with one-day-old samples, except in the cases specified otherwise.

Particle Size Analysis Microsphere size was determined with sieve analysis (AS200 Analytical Sieve Shaker, Retsch GmbH & Co. KG, Germany). Sieves with mesh sizes 630, 500, 400, 315, 250, 200, 160, 125, 100, 80, 63, and 50 μm were used. Sifting time was 20 min.

Drug Content Determination A known quantity of microspheres (*ca.* 10 mg) was dispersed in 96% ethanol and stirred for an hour. A small volume of the sample was filtered, diluted with ethanol, and the ketoprofen concentration was determined spectrophotometrically at 256 nm (Mettler TOLEDO AB54-S, Swiss).

Ketoprofen Release Studies The USP XXVI paddle method was used to determine of ketoprofen release kinetics. Approximately 80 mg of microspheres were dispersed in 1 l of phosphate buffer saline pH 7.4 (Ph. Eur. 3rd Ed.) and stirred at 100 rpm at 37 °C for 6 h. Samples were taken at 5, 10, 15, 30, 60, 120, 180, 240, 300 and 360 min and ketoprofen concentration was determined spectrophotometrically at 260 nm. We have studied ketoprofen release from whole samples (only a fraction of particles agglomerates bigger than 630 μm, which had also been excluded from the mean particle size calculation, was cast aside) and from the size fraction 160—200 μm. Drug release profiles were compared by MANOVA using all experimental sampling points.

Microscopy A scanning electron microscope (JEOL JSM 5800) was used to determine microsphere shape and surface characteristics. Prior to examination, the samples were carbon-coated and spattered with gold. Accelerating voltage was 14 kV.

High Sensitivity DSC High sensitivity differential scanning calorimetry (HSDSC) was performed using a Setaram Micro DSC III. Closed stainless steel batch vessels were used. Sample weights of approximately 8 mg were loaded into the sample vessel, whereas the reference vessel was left empty. A heating interval from 20 to 110 °C and a heating rate of 0.1 K/min were used. Thermograms of one-day-old samples and samples of various ages (1 month, 1 year, 2 years) were taken in triplicates with very good reproducibility. The samples were aged at uncontrolled room conditions in closed glass containers. Additionally, magnesium stearate content was determined by the HSDSC method, described in detail in ref. 14.

X-Ray Diffractometry XRD powder patterns were obtained with a Huber Guinier camera, using Cu$K\alpha$ radiation and a quartz monochromator. Microspheres, powdered samples of individual components used for microsphere preparation, and their physical mixture were analysed. The analysed microspheres were one year old and were aged at uncontrolled room conditions in closed glass containers.

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Results and Discussion

Particle Size Determination For each batch of microspheres, a sieve analysis was performed. The results are shown in Table 1 and Fig. 1, where it can be seen that preparation temperature has a strong effect on microparticle size, at least in the temperature interval studied. Microspheres with a higher average particle size and wider particle size distribution are formed when a higher preparation temperature is used. Significance of this influence on both parameters has been statistically proven by one-way ANOVA ($p<0.0001$).

These findings are in accordance with the above mentioned studies.[2,5] On the other hand, our previous study reports the insignificant influence of preparation temperature on microsphere properties.[3] We suppose that in that case the temperatures (40, 50, 60 °C) were too high to produce any significant differences. This is a justified speculation if one considers the mechanism of microparticle formation and the role of temperature in it. After emulsification of both emulsion phases, the inner solvent diffuses into the outer one and evaporates from the system. Higher preparation temperatures increase the rate of solvent removal, as they decrease the viscosity and increase the overall permeability coefficient of the inner-phase solvent.[4] At higher temperatures emulsion droplets harden faster. They may not "have time" to be influenced by stirrer shear forces, which tend to minimize droplets. A temperature of 40 °C causes a very rapid solvent removal; thus microsphere size distribution reflects the droplets size distribution in the initial emulsion just after combining both phases. It is therefore understandable that in this case particle distribution is very wide. On the other hand, at lower temperatures there is more time for intensive emulsification to take place and reach small droplets with uniform size distribution.

Table 1. The Effect of Preparation Temperature on Mean Particle Diameter and Magnesium Stearate Content in Microspheres

Preparation temperature (°C)	Mean particle diameter (μm) ±S.D.	Mg-stearate content (%) ±S.D.
10	94.0±3.1	12.6±0.2
25	116.5±3.3	9.4±0.5
35	174.2±9.7	8.1±0.3
40	238.1±30.6	7.9±0.1

Standard deviations (S.D.) of three determinations are presented.

Microscopy Figure 2 shows SEM micrographs of microspheres prepared under different temperature conditions: 10, 25 and 40 °C. Some morphological characteristics, *e.g.* diameter, shape of particles and surface characteristics, can be observed as well as their dependence on preparation tem-

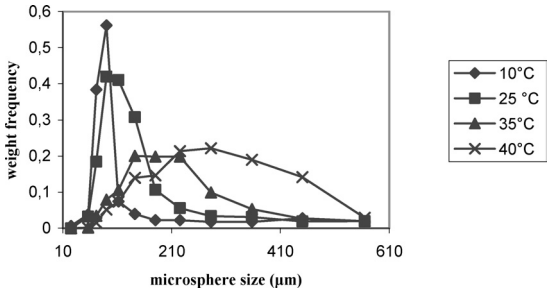

Fig. 1. Particle Size Distributions of Microspheres Prepared at Different Temperatures

Means of three determinations are shown.

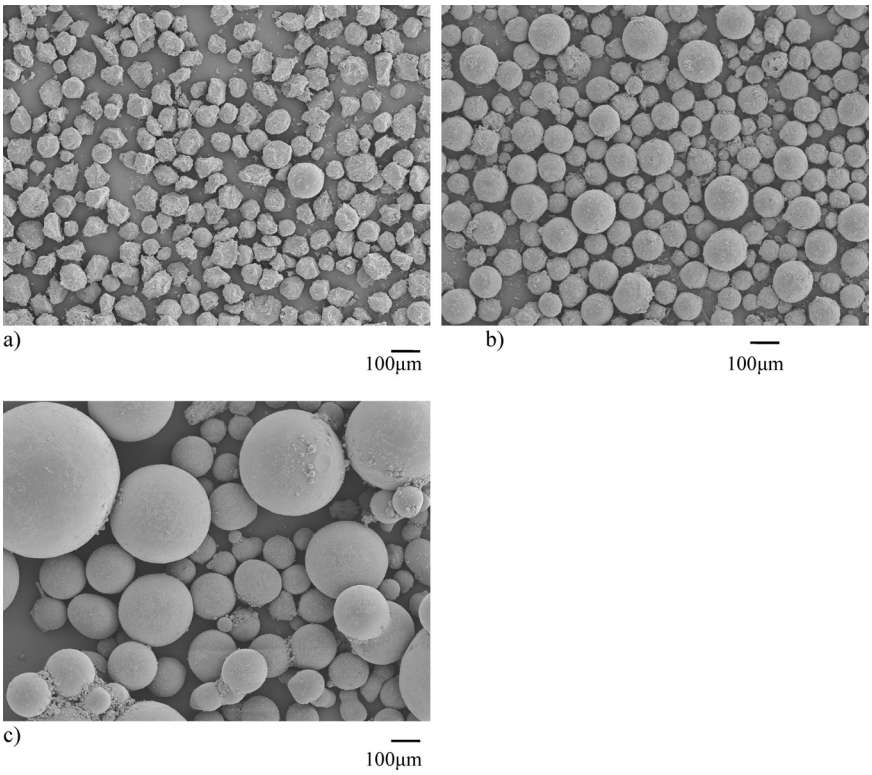

Fig. 2. SEM Photographs of Microspheres Prepared at Various Temperatures

a) 10 °C, b) 25 °C, c) 40 °C.

perature. At lower temperatures particles do not possess regular spherical shape; as temperature rises their shape becomes more and more spherical. With rising temperature surface characteristics change as well. Particles produced at 10 °C have a rough, wrinkled surface, with smaller irregular particles adhered on it. With increasing temperature the surface becomes smoother and adhered particles more spherical.

The effect of temperature on particle shape can also be explained by solvent removal rate and differences in continuous phase. We had problems with microspheres isolation from liquid paraffin at 10 °C because dispersion was so viscous. We presume that high viscosity is also the reason for irregular shape of the microspheres formed at 10 °C. Because of slower removal of solvent from the inner phase the polymer solution (droplets) changes slowly into more and more viscous phase, where a droplet is very susceptible to mechanical stress. When the stirring paddle hits the emulsion droplet, it deforms it, but irregular shape normally quickly changes into thermodynamically more favorable spherical shape. However, when viscosity is high the process is slowed and solidification may occur while the highly viscous droplet is deformed. Thus, hardened microspheres are of irregular shape.

Drug Encapsulation We have tested drug encapsulation efficiencies for all batches of microspheres and for fraction with diameter of 160—200 μm in each batch. Encapsulation efficiencies were all very high—over 95%. There is no difference in encapsulation efficiency between different batches, which is understandable as ketoprofen has a low solubility in liquid paraffin.

HSDSC Thermograms of microspheres prepared at 10 and at 40 °C and of their pure components are shown in Fig. 3. The quantities of pure substances are the same as they are in microspheres. The HSDSC study reveals that encapsulated ketoprofen is not in crystalline form, neither in the case of fast solidification (high temperature) nor in the case of slow solidification (low temperature), as no melting peak is present. Palmieri[15] reports on the extremely slow crystallization of ketoprofen from melt, and it is possible that in our case drug crystallization is also slow. We assume that encapsulated ketoprofen is in amorphous state, which is in accordance with the report of El-Kamel *et al.*[16] More interesting is the fact that even one-year-old and two-year-old microspheres show the same HSDSC signal, which means that no crystallization occurred in that time.

X-Ray Diffraction The non-crystalline state of ketoprofen in microspheres was confirmed by XRD. Figure 4 presents XRD patterns of pure ketoprofen and of a physical mixture of all the solid components, used for microsphere preparation. The physical mixture shows the same pattern as pure ketoprofen, only with lower intensity, which means that ketoprofen crystallinity is not affected solely by mixing with other components. On the other hand, the microspheres show no pattern, thus confirming the amorphous state of ketoprofen. The analyzed microspheres were one year old; on the basis of HSDSC findings, we could suppose that two-year-old microspheres contain an amorphous drug as well.

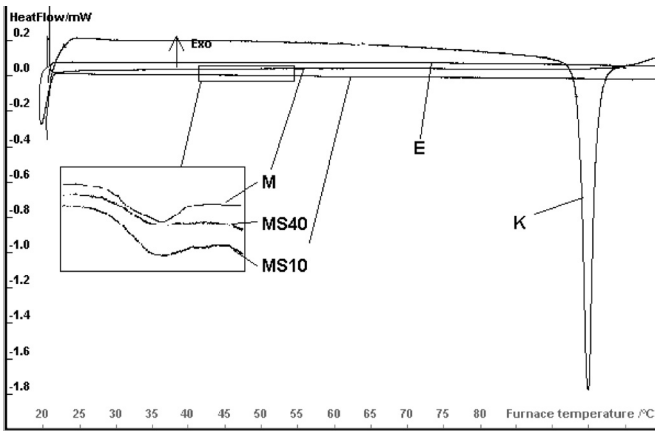

Fig. 3. Thermograms of Pure Ketoprofen (K), Magnesium Stearate (M) and Eudragit RS (E), and of Microspheres Prepared at 10 °C (MS10) and at 40 °C (MS40)

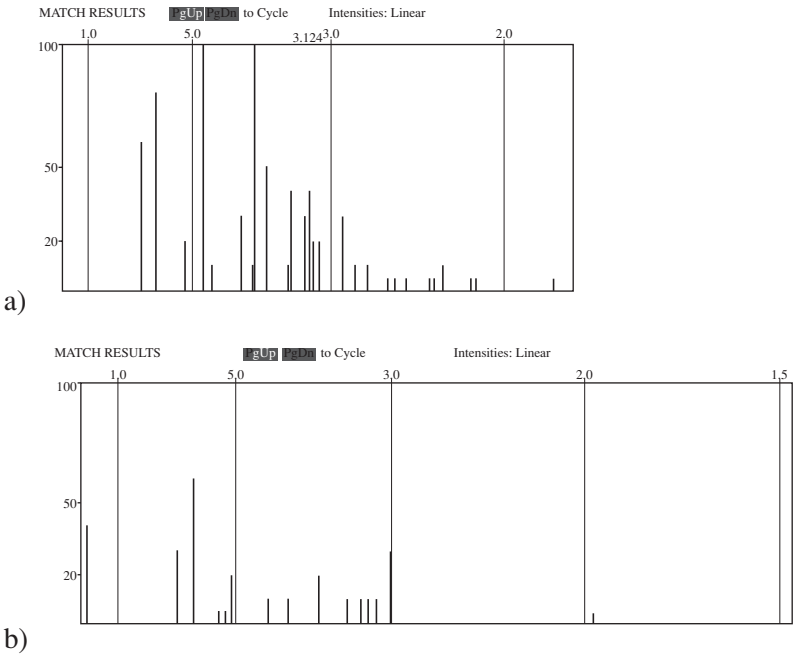

Fig. 4. X-Ray Diffractograms of Pure Ketoprofen (a) and Physical Mixture of all Components Used for Microspheres Preparation (b)

Magnesium Stearate Determination The only signal present in thermograms of microspheres belongs to the magnesium stearate—a droplet stabilizer, which forms a film of solid particles on the droplet surface and thus mechanically prevents their coalescence. A HSDSC method for stearate quantification in mixture with Eudragit RS was developed[14] and the content of encapsulated magnesium stearate was determined. The results are presented in Table 1, wherefrom it is obvious that the content of the stearate decreases as the temperature increases. It is possible that with increasing temperature the solubility of magnesium stearate in liquid paraffin increases and it diffuses into the outer emulsion phase to a greater extent. These results are in good accordance with our previous report.[3]

Drug Release Drug release from microspheres is influenced by the particle mean diameter and distribution width, by particle porosity, by the homogeneity of drug distribution within microparticles, *etc*. In order to account all those influences, we have studied ketoprofen release from whole samples; on the other hand we wanted to avoid the effect of different mean diameters and distribution widths, so ketoprofen release was also studied from the size fraction 160—200 μm. The results of the drug release profile from the whole sample are shown in Fig. 5a and the results of release from fraction 160—200 μm in Fig. 5b. We expected the release rates to correlate with the specific surface area due to particle size and porosity.

If we observe drug dissolution from the whole samples, we see that this correlation does exist. The overall effect of temperature on the drug release profile was proved by MANOVA to be significant ($p_{\text{Pillai's Trace}}<0.05$).

The size fraction 160—200 μm shows similar results. The overall effect of temperature on the drug release profile was proved to be significant ($p_{\text{Pillai's Trace}}<0.05$). We suppose that the differences in release profiles are the consequence of differences in the surface area due to particle surface roughness and porosity. On the basis of comparison of the dissolution profiles from whole samples and from chosen size fraction one could suppose that particle surface characteristics a play more pronounced role than particle size.

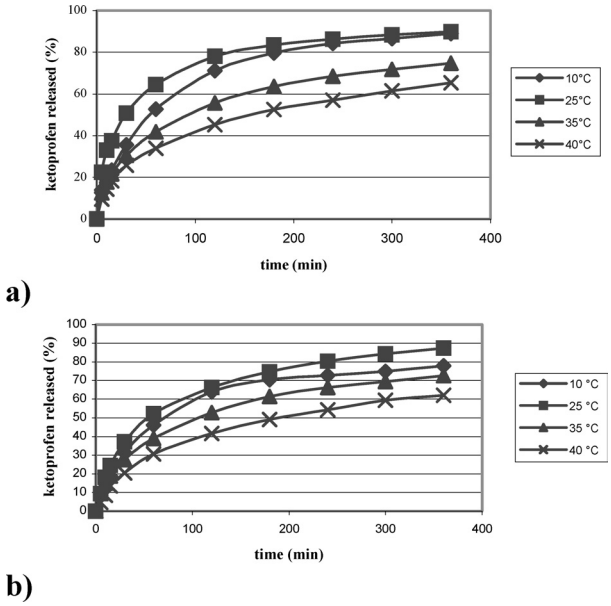

Fig. 5. Drug Release Profiles from Microspheres Prepared at Different Temperatures

a) Whole sample, b) size fraction 160—200 μm.

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