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Preparation of microcapsules with self-microemulsifying core by a vibrating nozzle method

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Incorporation of drugs in self-microemulsifying systems (SMES) offers several advantages for their delivery, the main one being faster drug dissolution and absorption. Formulation of SMES in solid dosage forms can be difficult and, to date, most SMES are applied in liquid dosage form or soft gelatin capsules. We have explored the incorporation of SMES in microcapsules, which could then be used for formulation of solid dosage forms. An Inotech IE-50 R encapsulator equipped with a concentric nozzle was used to produce alginate microcapsules with a self-microemulsifying core. Retention of the core phase was improved by optimization of encapsulator parameters and modification of the shell forming phase and hardening solution. The mean encapsulation efficiency of final batches was more than 87%, which resulted in 0.07% drug loading. It was demonstrated that production of microcapsules with a self-microemulsifying core is possible and that the process is stable and reproducible.

Keywords: Self-microemulsifying systems, vibrating noozle, microcapsules, ketoprofen

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Introduction

In recent years more and more active ingredients are known which exhibit low bioavailability due to their low solubility. Application of such drugs in lipid vehicles can greatly increase their bioavailability and there are some products already on the market. However, use of pure lipid carriers is not suitable for most drugs, although special substances, such as vitamins A and D, have been delivered in this way for a long time (Humberstone and Charman 1997). The formulation of emulsions, microemulsions and other similar systems with lipids can greatly enhance bioavailability of many substances (Pouton 2000, Kang *et al.* 2004). Microemulsions have many advantages over emulsions, including thermodynamic stability, greater drug solubilization capacity and permeability enhancement. Because their droplet size is smaller than that of emulsions, they have a greater specific surface area and drug dissolution from microemulsions is consequently faster. In addition, the intra- and inter-patient variability of pharmacokinetic parameters is reduced when a drug is administered in form of a microemulsion. Formulation of drug delivery systems based on self-emulsifying (SES) and self-microemulsifying (SMES) systems which, after application, form emulsions and microemulsions in aqueous physiological media, offers additional advantages. The most important ones are their stability that is greater than that of emulsions or even microemulsions, and their greater drug loading capacity (Gershanik and Benita 2000, Kommuru *et al.* 2001, Holm *et al.* 2003).

Microparticulate drug delivery systems, such as microcapsules and microspheres (or pellets), are widely used in pharmacy for a number of applications such as controlled peroral delivery. Their advantages over single unit drug delivery systems are absence of dose dumping and larger surface area. The use of biodegradable substances, such as alginate and chitosan, has further benefits for safety (Kas 1997, Freiberg and Zhu 2004). Microcapsules are micrometer-sized particles (20-2000 μm), outwardly similar to microspheres, but with a distinguishable core and shell. The active ingredient is generally located in the core and the shell is usually formed from polymeric material. A vibrating nozzle method is often used for alginate bead preparation, since it allows high production rate of uniform sized beads with mean diameter below 300 μm. The process can be carried out under mild, nontoxic conditions and can easily be scaled up. Because it also allows complete sterility of the process, present day applications are mostly oriented towards cell encapsulation (Serp *et al.* 2000, Koch *et al.* 2003).

Application of SMES and SES is mostly limited to liquid dosage forms and soft gelatin capsules, which are not optimal formulations for peroral application. Inclusion of such systems in microcapsules would greatly broaden formulation possibilities, most notably allowing the formulation of solid dosage forms. The purpose of our work was to prepare microcapsules with SMES in the core, which would be appropriate for further use in the production of solid peroral dosage forms. We have used an Inotech IE-50 R encapsulator with binary nozzle to produce microcapsules with a liquid core. Alginate solution with lactose was used as the shell forming solution, while SMES loaded with ketoprofen was used as the core forming phase. Prepared microcapsules were dried in a fluid bed system.

Materials and methods

Materials

Self-microemulsifying systems were prepared using Miglyol 812® (CONDEA Chemie Gmbh, Witten, Germany) as lipophilic phase and Labrasol® and Plurol oleique® (GATTEFOSSE, Saint-Priest, France) as surfactant and co-surfactant respectively. Sodium alginate with low viscosity was from Sigma (Germany). Polyethylene glycol (PEG) 10,000 and 20,000 were obtained from Fluka (Germany). Ketoprofen and lactose (mesh 200) were donated by Lek pharmaceutical company. All other chemicals were of laboratory grade.

Methods

Preparation and evaluation of self-microemulsifying systems

All self-microemulsifying systems (SMES) were prepared by mixing surfactant and co-surfactant in a ratio of 4:1 using a magnetic stirrer. The ratio was determined in previous work with microemulsions (Špiclin *et al.* 2003). A range of quantities (2.5, 10, 20, 30 and 40%) of Miglyol were added to the surfactant mixture and briefly mixed to give homogeneous mixtures. The loading capacity of ketoprofen in each SMES system and in surfactant mixtures alone was determined by means of UV absorbance (Hewlett Packard 8453 Spectrophotometer) at 255 nm. Methanol was used to dilute the systems before measurement. All ketoprofen-loaded systems were shown to be stable over a period of 3 months (data not shown). The most suitable ketoprofen-loaded system was used for preparation of microcapsules.

Production of microcapsules

An Inotech IE-50 R encapsulator equipped with a 150µm/250 µm concentric nozzle and two 50 ml syringes was used to produce microcapsules with a self-microemulsifying core. Water solutions of Na-alginate containing different excipients were used as the shell forming phase and a self-microemulsifying system containing 10 % of ketoprofen was used as the core forming phase. Microcapsules were produced at optimum flow rates of the two phases, vibration parameters and electrostatic dispersion settings. Microcapsules were incubated in hardening solution for 30 minutes, rinsed with water and dried on transparent paper (Carta Ciat – Lucido, Ciat, Italia). Several hardening solutions were tested (0.3 M $CaCl_2$, 0.3 M $AlCl_3$, 1 mg/ml chitosan, 0.3 M $CaCl_2$ with 1 mg/ml chitosan, and a 0.3 M mixture of $CaCl_2$ and $ZnCl_2$ in ratio 1:1). The pH of all hardening solutions was adjusted with 0.01 M HCl to pH 3 to ensure proper solubilization of chitosan. A 0.3 M $CaCl_2$ hardening solution with unadjusted pH (pH = 7.3) was used to determine the effect of pH.

Microcapsules produced at optimum parameters and hardening solution were spread on transparent paper and allowed to drain for 20 minutes before drying in a fluid bed system (Strea 1, Niro Aeromatic) equipped with a modified top spray vessel. Each batch of microcapsules, weighing approximately 60 g, was dried at an inlet air temperature setting of 55 °C until the outlet air temperature reached 47 °C. The volume of fluidizing air was regulated in the interval from 43 to 115 m^3/h to ensure optimum fluidizing of microcapsules. Drying was stopped several times in order to break apart larger agglomerates of microcapsules and to detach microcapsules sticking to vessel sides.

Optical analysis of microcapsules

Shape and surface morphology as well as core position of freshly prepared and dried microcapsules were observed under an Olympus SZX 12 optical microscope mounted with a Sony 950P digital camera. Pictures were taken of microcapsules after 1 and 30 minutes of incubation in hardening solution and also of dried microcapsules.

Determination of ketoprofen content and entrapment efficacy

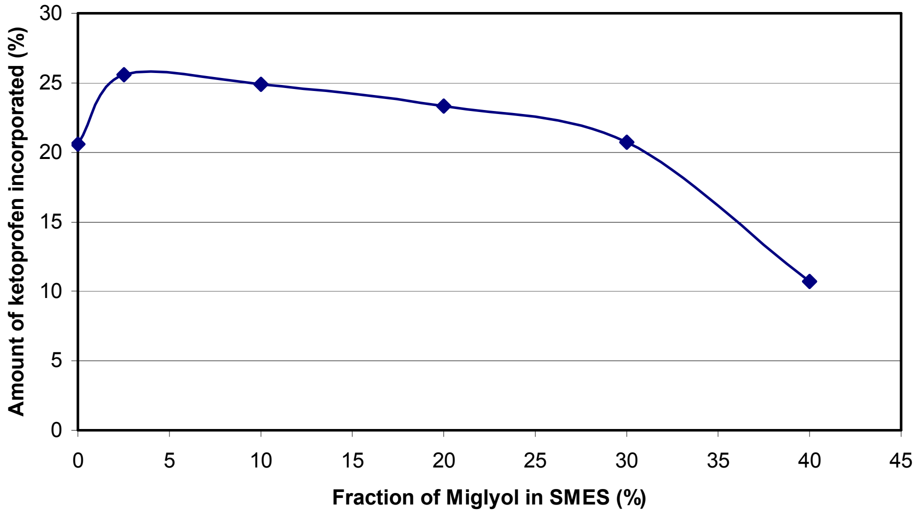
The amount of incorporated ketoprofen was determined in freshly produced and in dried microcapsules. Microcapsules were crushed and incubated in NaOH solution at pH 9 for 24 hours. After incubation the mixture was sonicated for 15 minutes, centrifuged at 3000 rpm for 15 minutes (LC-321, Tehtnica, Slovenia) and the supernatant filtered through a 0.22 μm cellulose acetate filter (Sartorius, Germany). If needed, the samples were diluted with purified water prior to absorbance measurement. The concentration of ketoprofen was determined by absorbance measurement at 260 nm. Microcapsules without ketoprofen, produced under the same operating parameters and treated in the same way, were used to set the baseline to zero. Entrapment efficacy was calculated as the amount of encapsulated ketoprofen relative to the total amount of ketoprofen in the microcapsules, hardening solution and water used for rinsing.

Results and discussion

Evaluation of self-microemulsifying systems

The composition of self-microemulsifying systems (SMES) was optimized, with respect to ketoprofen loading capacity, for incorporation into microcapsules. Loading capacity should be as high as possible, since this allows more drug to be incorporated into the formulation. Figure 1 shows the maximum ketoprofen loading capacity as a function of the fraction of Miglyol in SMES. The loading capacity increases with decreasing Miglyol content. However, Miglyol is required for high ketoprofen loading capacity since, in a comparable mixture of surfactants without Miglyol, loading capacity is significantly lower than in mixtures with up to 20% Miglyol. From the practical viewpoint, formulation of SMES with 20-30 % of Miglyol is rational, since loading capacity for ketoprofen is only slightly decreased. Higher Miglyol content means lower surfactant and co-surfactant content, which is preferable since surfactants are the main cause of undesired side effects. SMES have been shown to have comparable drug release rates to those of microemulsions prepared from SMES (Homar *et al.* 2005), indicating that microemulsions are formed as soon as SMES comes into contact with aqueous media. On the basis of the results, SMES with 20 % Miglyol content were selected for the production of microcapsules. SMES with this composition have relatively low surfactant content but high drug loading capacity, which are both required for an SMES intended for incorporation in microcapsules.

Figure 1: Ketoprofen loading capacity of SMES as a function of Miglyol content.



Determination of optimal process parameters

All microcapsules were prepared with an Inotech IE-50 R encapsulator equipped with a 150μm/250 μm concentric nozzle. The shell forming phase envelopes the core forming phase as they flow simultaneously through the nozzle, forming a continuous jet that is broken apart into droplets by vibration of the membrane. When these droplets come in contact with hardening solution, a sol–gel transition of alginate in the shell occurs, encapsulating SMES with the drug in the core. SMES loaded with 10% ketoprofen was used as core forming phase, and 2.0% sodium alginate solution as shell forming phase.

A continuous and stable flow of shell forming phase was reached at a speed setting of 6.019 ml/min. The speed could be increased up to 8.973 ml/min, when safety shutdown of the machine prevented further increase. The interval of shell forming phase speed setting was independent of core forming speed setting variations. The flow rate of core forming phase could be increased from 0 to 0.772 ml/min, regardless of the speed setting of the shell forming phase, before safety shutdown of the machine occurred. It is desirable that the core forming phase flow rate is high, since this allows a greater concentration of drug to be incorporated into microcapsules. A speed setting of 0.057 ml/min for the core forming phase was selected for all further experiments, since this was the highest setting that allowed an overall stable process with a small safety margin, which allowed slight modifications. If the shell forming phase flow rate is high, a thicker shell is produced, resulting in increased mechanical strength of the microcapsules and core phase retention. On the other hand, high flow rate of the shell forming phase reduces the proportion of core phase and consequently the amount of drug that can be incorporated into microcapsules. Considering this, we used a shell forming phase flow rate of 7.119 ml/min for all further experiments. Microcapsules produced at these settings showed relatively good core phase retention and position, low size variation and good reproducibility.

The vibration of the membrane can be controlled by adjusting the amplitude and the frequency of vibration. Previous experiments showed that the amplitude setting has very little influence on the size and quality of produced microcapsules, therefore it was kept constant at a medium value throughout all experiments. Microcapsules were prepared at membrane frequency settings of 196, 550, 1000 and 1400 Hz. Surprisingly, variation of frequency did not substantially influence the size of microcapsules. Only a small increase in size was observed when the frequency was decreased. With decreasing frequency the uniformity of size distribution also increased, therefore a frequency of 550 Hz was used in further experiments.

During production, microcapsules showed a tendency to agglomerate when the electrostatic dispersion unit was switched off. Therefore, the voltage setting of the electrostatic dispersion unit was varied in the interval 0.5 to 1.80 kV. Good dispersion of droplet chain was observed at all settings and all consequent microcapsules were produced at a voltage of 1.5 kV.

Modification of the shell forming phase

A shell forming phase consisting solely of sodium alginate showed leakage of core phase during preparation as well as from completed microcapsules. Increasing the concentration of sodium alginate in increments of 0.5% from 1.0 to 2.5% (w/w), led to no great improvement of core phase retention. Further increase in sodium alginate concentration was not possible, since the encapsulator could not process such viscous solutions. Modification of the shell forming phase was therefore necessary in order to reduce core leaking. Polyethylene glycol, sucrose, sorbitol, lactose and NaCl were added to sodium alginate solution in different concentrations and combinations to address this problem. The choice of excipients was based on the hypothesis that the addition of hydrophilic molecules would reduce core leakage by decreasing the mobility of water molecules and by filling the pores in dried alginate gel. The addition of these substances to shell forming phase containing 2.0% or more of sodium alginate resulted in viscous solutions, which were very hard to process, therefore solutions with 1.5% sodium alginate were used. The volume of hardening solution (80 ml) and the amount of prepared microcapsules (20 g) were constant throughout all experiments. Reduction of core leakage was assessed by optical inspection of the turbidity of hardening solution after the preparation of microcapsules. Three different levels of turbidity, high, medium and low, were distinguished. Higher turbidity signified greater core leakage, since the core phase formed a milky emulsion on mixing with hardening solution. The composition of the shell forming phase and corresponding turbidity of hardening solution are shown in Table 1.

Table 1: Composition of shell forming phase and turbidity of hardening solution.

Alginate conc. (w/w)	Additional excipients (w/w)	Turbidity
1,5%	5% PEG 4000	High
1,5%	10% PEG 4000	High
1,5%	20% sucrose	High
1,5%	7% sorbitol	Moderate
1,5%	5% lactose	Low
1,5%	10% lactose	Low
1,5%	5% lactose + 5% sorbitol	Low
1,5%	5% lactose +15% NaCl	No microcapsules formed

We observed that the addition of excipients which increase the viscosity of shell forming phase generally reduces core phase leakage during microcapsule preparation. However, the increase of viscosity narrows the interval of optimal flow rates, until at a certain viscosity the production of microcapsules is no longer possible. Viscosities close to the upper viscosity limit of the encapsulator processing capability were therefore used. The addition of hydrophilic materials to the shell forming phase confirmed our speculation that core phase leakage can be decreased during preparation due to interaction of these molecules with water molecules. Because of these interactions the mobility of water molecules is reduced, thus preventing rapid mixing of shell forming phase with self-microemulsifying core. Added excipients are also expected to fill pores in dried alginate gel and thus reduce core leakage from dried microcapsules. This was confirmed by improved retention of core phase on drying the microcapsules when different excipients were added to the shell forming phase. The retention was comparable for all excipients used and did not change much when the concentration of the excipients was increased above 5%, regardless of the molecular mass of the excipient.

Addition of sodium chloride to the shell forming phase resulted in uncontrolled microcapsule spraying after the stream of microcapsules passed through the electrostatic dispersion unit, regardless of voltage setting. If the unit was not used, the process resulted in lumps of alginate. Addition of PEG 4000 showed poor core phase retention, although these solutions were the most viscous ones. Sucrose also showed poor core phase retention, while sorbitol showed a slight improvement. Of all excipients tested, lactose showed the greatest reduction of core leaking. Addition of 5% (w/w) was enough to visually reduce turbidity. Increase of lactose concentration or addition of sorbitol did not improve the retention of core phase.

Modification of hardening solution

Change of hardening solution offers a relatively simple way of modifying shell structure. It is known that different ions interact in different ways with alginate, producing gels with different properties. The valence of ions is mostly the reason for different cross-linking of alginate and hence for different shell properties. However, ions of the same valence can also interact differently with alginate molecules resulting in different shell properties (Aslani and Kennedy 1996, Gonzalez-Rodrigez *et al.* 2002, Chan *et al.* 2002).

Hardening solutions with $CaCl_2$, $AlCl_3$, chitosan, $CaCl_2$ with chitosan, and a mixture of $CaCl_2$ and $ZnCl_2$ in ratio 1:1 were prepared. All hardening solutions had a pH of 3 and a concentration of hardening ions of 0.3 M, with the exception of chitosan solutions, whose concentration was 1 mg/ml. The volume of hardening solution (80 ml) and the mass of prepared microcapsules (20 mg) were constant throughout all experiments. Preliminary experiments showed that alginate microcapsules prepared with hardening solution consisting solely of zinc ions tend to agglomerate during the hardening process, therefore this hardening solution was not used further.

Microcapsules prepared with hardening solution containing chitosan had poor mechanical stability and were therefore excluded from further studies. Microcapsules prepared with hardening solution containing $AlCl_3$ and $CaCl_2$ with chitosan showed increased porosity and leaking during preparation and drying and were also excluded from further studies. Since the retention of core phase for microcapsules hardened with $CaCl_2$ solution and $CaCl_2/ZnCl_2$ solution could not be successfully distinguished by the turbidity of hardening solution, we used the ketoprofen content as the criterion. Microcapsules hardened with $CaCl_2$ solution contained 0.0253% of ketoprofen and microcapsules hardened with a mixture of

$CaCl_2$ and $ZnCl_2$ solution contained 0.0177% of ketoprofen. Encapsulation efficiency was 23% and 18 % respectively. We conclude that, of all hardening solutions tested, $CaCl_2$ solution showed the best core phase retention.

All hardening solutions used were adjusted to pH 3. Since ketoprofen is poorly soluble in acidic conditions, the influence of pH of the hardening solution on ketoprofen retention was assessed. $CaCl_2$ solution with unadjusted pH (pH = 7) was used to determine the influence of pH on ketoprofen content. Microcapsules produced with this hardening solution contained 0.0124% of ketoprofen and the entrapment efficacy was 14%. Decreasing the pH of the hardening solution clearly retards the release of ketoprofen during preparation.

Based on our findings all subsequent microcapsules were prepared with 0.3 M $CaCl_2$ solution adjusted to pH 3.

Drying microcapsules

Three sets of approximately 20 g of microcapsules were prepared in order to evaluate process reproducibility and stability. Core phase flow speed was set to 0.057 ml/min and shell forming phase flow speed to 7.119 ml/min. SMES with 10% ketoprofen was used as core forming phase and 1,5% sodium alginate solution with 5% lactose was used as shell forming phase. Hardening solution contained 0.3 M $CaCl_2$, pH 3.

Table 2 shows entrapment efficacy and ketoprofen content for each batch tested. Mean ketoprofen content in freshly prepared microcapsules was 0.0239% with mean entrapment efficacy of 22.47%. Entrapment efficacy was relatively low, indicating that leakage of core phase was still present during microcapsule preparation. The relative standard deviation (RSD) of both variables was below 4%, indicating good process reproducibility and stability. All produced microcapsules showed small size variation and were round to oval in appearance (Figure 2a).

Table 2: Ketoprofen content and entrapment efficacy of freshly prepared microcapsules.

Sample No.	Ketoprofen content (%)	Entrapment efficacy (%)
1.	0.0242	23.37
2.	0.0241	22.33
3.	0.0235	21.72
Mean	0.0239	22.47
RSD (%)	1.58	3.71

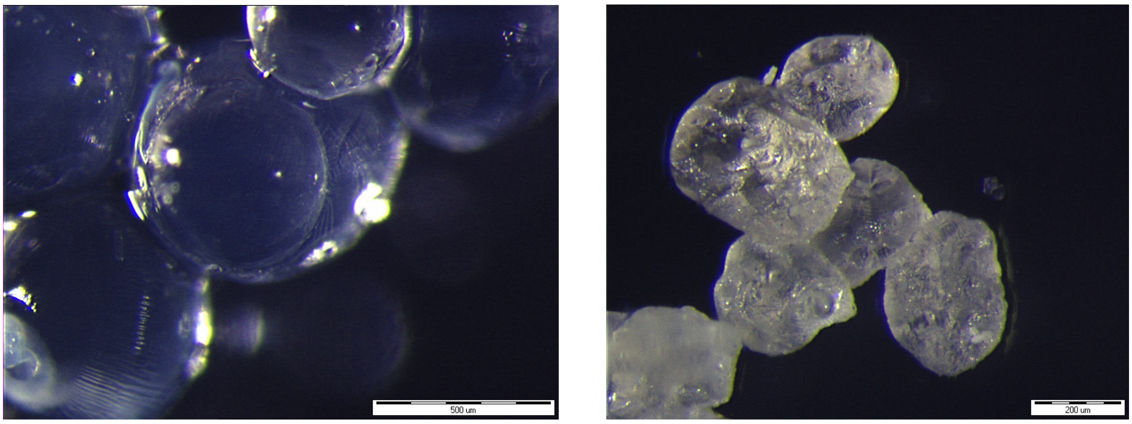
Three sets of approximately 60 g of microcapsules were prepared with the same parameters as above in order to assess process reproducibility and stability after drying. The drying process lasted from 15 to 20 minutes, depending on the number of interruptions made, during which microcapsules were detached from vessel sides and larger agglomerates were broken apart. Microcapsules size and weight were greatly reduced during drying, nevertheless they retained a roughly spherical form (Figure 2b). Mass loss was determined to be 93% of initial mass and was attributed to water evaporation. Table 3 shows entrapment efficacy and ketoprofen content for each batch tested. Mean ketoprofen content in dried microcapsules was 0.0684% with mean entrapment efficacy of 87.49%. Relative standard deviation was 5% and 11% for ketoprofen content and entrapment efficacy respectively.

Table 3: Ketoprofen content and entrapment efficacy of dried microcapsules.

Sample No.	Ketoprofen content (%)	Entrapment efficacy (%)
1.	0.0650	76.15
2.	0.0719	92.20
3.	0.0683	94.13
Mean	0.0684	87.49
RSD (%)	5.04	11.28

Entrapment efficacy of 60 g batches was much higher than that for microcapsules prepared in 20 g batches, probably due to saturation of hardening solution with ketoprofen. The volume of hardening solution in both cases was constant at 80 ml. Surprisingly, ketoprofen concentration increased only 3-fold, although at least 14-fold increase was expected due to mass reduction and higher entrapment efficacy. Ketoprofen loss probably occurred during draining of microcapsules, before they were placed in the fluid bed system. Relatively high standard deviations of both variables can also be ascribed to ketoprofen leakage.

Figure 2: Microcapsules before (A) and after drying (B), filmed at magnification 80 and 100 times, respectively.



A B

Conclusions

In this work we have shown that production of dry microcapsules with SMES in the core is possible. Using an encapsulator equipped with vibrating nozzle we have produced and subsequently dried microcapsules with modified alginate shell and self-microemulsifying core loaded with ketoprofen. Both shell and core compositions were optimized to minimize core leakage and drug loading capability, respectively.

The optimum amount of lipid phase in SMES was determined to be 20%, based on maximum ketoprofen loading capacity and surfactant/co-surfactant concentration. Process parameters for production of liquid core microcapsules were examined. We established that amplitude and frequency of the membrane had no significant effect on microcapsule properties. On the other hand, the electrostatic dispersion unit was required for successful preparation of microcapsules. Flow rates of inner and outer phase were found to be limited by the machine settings, consequently limiting the maximum amount of incorporated ketoprofen. Modification of the shell forming phase was necessary to reduce core leakage. Addition of 5% lactose was shown to greatly reduce core leakage. A hardening solution with Ca^{2+} ions and a pH of 3 was chosen as optimal. The process was shown to be stable and reproducible with microcapsules retaining a roughly spherical form on drying. Unexpected drug loss occurred during draining the microcapsules, but can be reduced by shortening the draining process. Overall, the production of dry microcapsules with SMES core was shown to be possible and reproducible.

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