Novel approach for characterization of microspheres made by solvent evaporation

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Introduction

The solvent evaporation encapsulation technique has widely been used for preparation of microspheres for controlled release of drugs. The drug trapped inside the microspheres releases once the microspheres are introduced into a suitable release environment. The operating conditions to prepare these micro spheres have to be optimized to obtain a desirable drug release profile. The preparation method consists basically of four major steps (Figure 1): (1) dissolution of polymer and active principle in organic solvent; (2) emulsification of this organic phase called dispersed phase in an aqueous phase called continuous phase; (3) extraction and evaporation of solvent; (4) rinsing and recovery of microspheres.

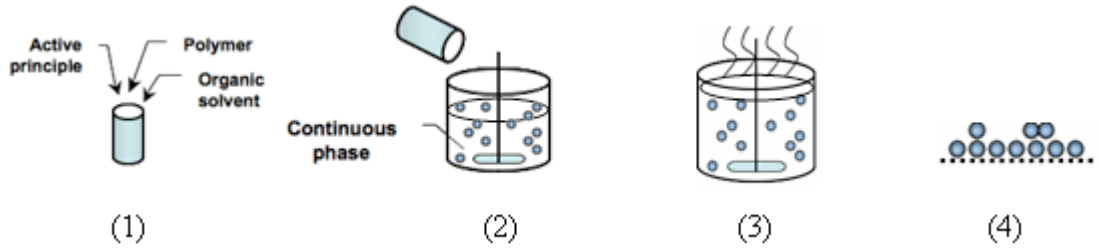


Figure 1: Basic steps of microencapsulation by solvent evaporation

A large number of publications focus on the choice of polymer and characterization of release rate in vitro and in vivo (Freiberg et al., 1994). Few papers deal with the physico-chemical and engineering aspects. Operating conditions such as ratio of the dispersed phase to the continuous phase, agitation speed, pressure and temperature have great influences on the solvent evaporation process and consequently on the structure of microspheres. Our work consists of using a novel approach to compare the quality of drug-free microspheres obtained under different operating conditions and thus getting a better understanding on the solidification of microspheres and formation of pores inside.

Materials and methods

Materials selection

Name of products	Producer	Advantages
Dichloromethane	Sigma Aldrich, Fluka	Solvent which dissolves most of the polymers; almost immiscible in water; high volatility and quite low boiling temperature
Ethyl cellulose	Ref 247499 Sigma Aldrich	Biocompatible and degradable, approved for pharmaceutical applications, low cost
Hydrolyzed polyvinyl alcohol (PVA)	87%-89% hydrolyzed Sigma Aldrich	Surfactant which gives more spherical microspheres

Table 1: Selection of materials in our work and the reasons for the choice

Manufacturing of microspheres: A cylindrical reactor (inner diameter of 10cm and volume of 800 mL) with temperature control was used for manufacturing of microspheres. The reactor was connected with a volumetric pump (Masterflex). It was possible to work at reduced pressure about 600mBar (closed reactor) and at atmospheric pressure (reactor with one opening). In both cases, the evaporation rate of solvent could be controlled by varying the pump flowrate. The following conditions were kept the same for the manufacturing of microspheres: the temperature of the continuous phase was maintained at 25°C and the agitation speed was 500 rpm; 20 mL of the dispersed phase (5% (w/w) of ethyl cellulose in dichloromethane) was dispersed in 200mL of the continuous phase (0.04% PVA solution). The pressure and solvent removal rate were varied:

1) The solvent was removed by evaporation at atmospheric pressure at 0.04g/min, 0.246 g/min and 0.634 g/min. The solvent evaporation rate was calculated from the measured mass change of the system.
2) Solvent was removed under reduced pressure of 600 mBar.
3) Microspheres were obtained by using extraction. The dispersed phase was emulsified in the continuous phase for 5 minute for formation of initial emulsion. The dispersed phase (in form of drops) were then moved to a very big quantity of water to achieve a very fast solidification (in several minutes). Its rate was estimated at the level 10 g/min.

With the same experiment, we observed always two kinds of microspheres: solid microspheres and hollow microsphere (20%-40% w/w). The latter ones were the gas bubbles with polymer membranes around. We are interested only in the solid microspheres.

Characterization of microspheres: Surface of microspheres and sectioned microspheres were observed by scanning electronic microscopy (Joel, JSM-6400F). The inner structure of microspheres was scanned by micro-computed tomography (at the Department of Chemical Engineering in University of Birmingham, UK). The average porosity of the microspheres and the distribution of porosity in different zone were measured. 3-D images of microspheres were reconstructed by software ANT and Recon (Skyscan, Belgium) giving an explicit visualization of distribution of pores. The size of microspheres was measured by an image analysis method with software named Visilog (Noesis, France).

Results and discussion

***Reduction of process duration*:** At atmospheric pressure, the evaporation rate reached the maximum 0.64 g/min with our experimental device while this rate was increased to 1.58 g/min by applying a reduced pressure of 600 mbar. So applying a reduced pressure significantly accelerates the solvent evaporation. However, the pressure has to be kept above the saturated vapor pressure of solvent even though lower pressure increases the evaporation rate, because the solvent boils under the saturated vapor pressure, the bubble formation destroys the microspheres, resulting in polymer filaments.

***Influence of evaporation on the morphology of microcapsules* :** The Figure 2 shows that on the surface the size of pores decreases and the number of pores per unit area increases with the increase of solvent removal rate. Viscosity of the dispersed phase increases with the decrease of the quantity of solvent. Therefore, high viscosity due to fast evaporation rate limits the growth and fusion of bubbles, resulting in a more dense structure. The pores appear at any pressure so low pressure is not a prerequisite for formation of gas bubbles. Formation of bubbles could be due to the air dissolved in the solvent and/or to solvent vapor.

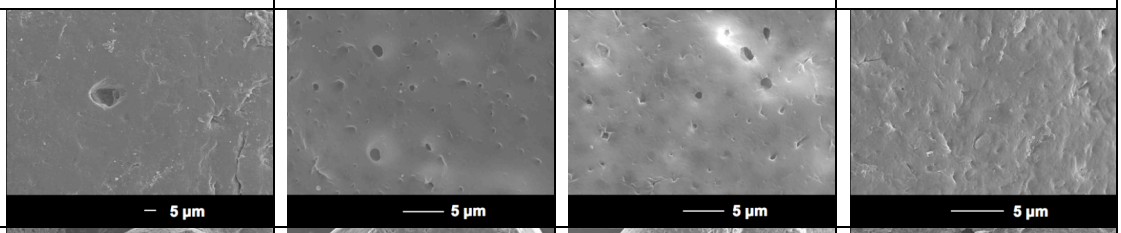
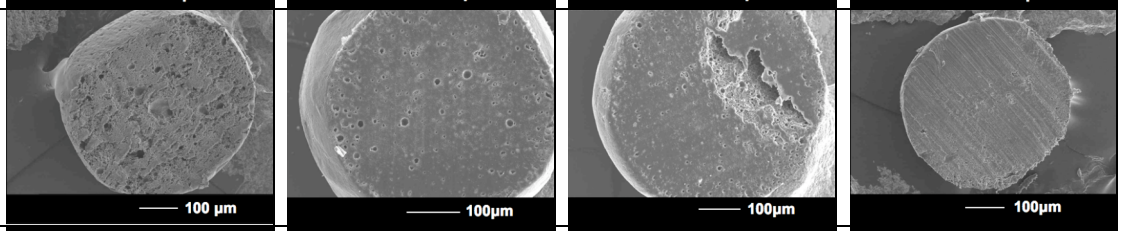
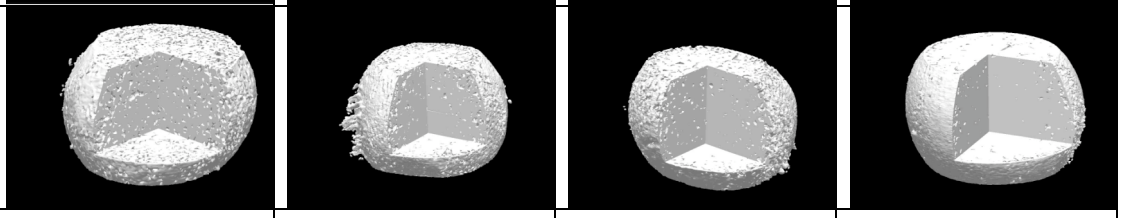
	Very slow evaporation	Slow evaporation	Fast evaporation	Extraction
Evaporation rate	0.004 g/min	0.246 g/min	0.634 g/min	≈ 10 g/min
Surface				
Section				
3-D reconstruction				
Pore's size on surface	< 14 µm	< 2 µm	< 2 µm	< 150 nm
Inner porosity	24%±2%	13%±1%	10%±1%	4%±0.5%
Pore's size inside	< 20 µm	< 35 µm	< 10 µm	< 3 µm

Figure 2: Photos and characterization of drug free microspheres made at atmospheric pressure at different solvent evaporation rate

The inner porosity decreases with increasing evaporation rate (Figure 3a). Microsphere is divided into 8 zones according to their distance to the center with constant interval equal to 1/8 of the radius. The porosity is very high near the surface of microsphere and decreases gradually in the direction to the center (Figure 3b). This is also confirmed by the 3-D reconstruction images in Figure 2. This observation is valid under all operating conditions. There are two possible hypotheses to be verified in the future work: 1) the formation of bubbles initializes near the surface; 2) bubbles moves to the surface.

Influence of pressure: Microspheres made under reduced pressure had generally smaller pores on the surface and inside the microspheres. Izumikawa et al. (1991) also observed smooth surface for microspheres made under reduced pressure. This observation is contrary to our initial expectation since the volume of pores should be bigger under low pressure according to the perfect gas law. There are two possible explanations: 1) solidification is much slower under atmospheric pressure, permitting the growth of the size of bubbles and the fusion of bubbles. This means that the evaporation rate has more impact on the porosity of microspheres than the pressure does; 2) Bubbles grow so big under reduced pressure that they cannot stay anymore stable inside the microspheres. Possibly due to gravity and centrifugal force, the bubbles leave the microsphere.

Therefore only bubbles of small size remain stable inside the microsphere and become a part of final structure of microsphere.

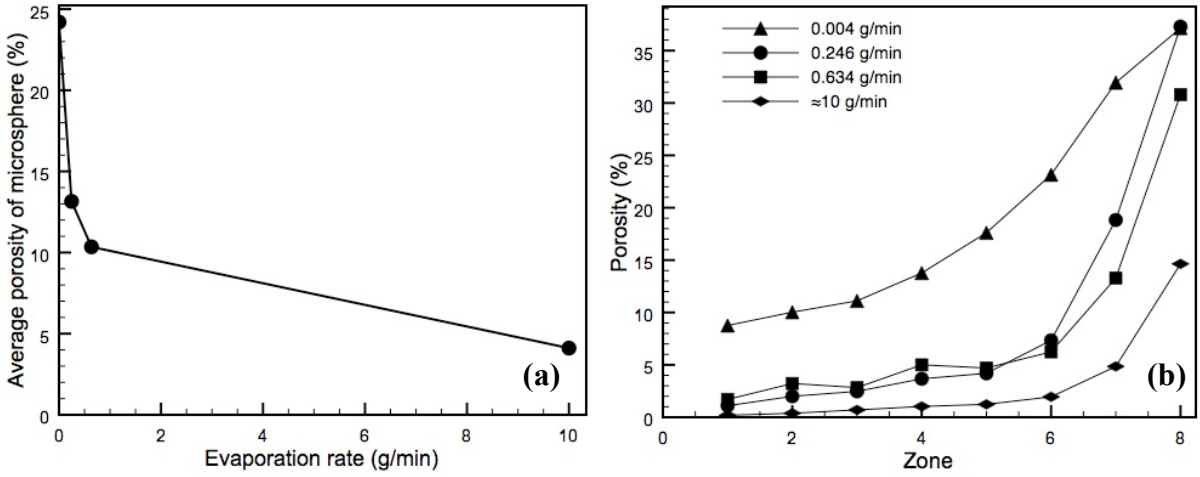


Figure 3: Average inner porosity of microspheres as a function of solvent evaporation rate (a) and porosity of different zones inside the microsphere at different solvent evaporation rates (b)

Microspheres made under atmospheric pressure have bigger average size of 647±50 μm than those made under reduced pressure of 460±70 μm. This difference is obviously not due to the difference of porosity since the average porosity of microspheres is only 5% and 2% respectively, neither due to the polymer loss (the same yield in both cases). The dispersion seems to be more efficient under reduced pressure. However, other reasoning for this size difference exists in the literature (Chuang et al. 2001), whose explanations are based on the Laplace equation.

Conclusions

Novel method of characterization by X-ray tomography has proven to be an efficient way to carry out a global and quantitative measurement of inner structure of microspheres. Combined with scanning electronic microscopy on the surface morphology and size measurement, most characteristics can be obtained. Reduced pressure increases significantly the solvent evaporation rate. Increasing evaporation rate decreases the size of pores on the surface and the inner porosity of the microspheres. Moreover, the porosity near the surface is very high compared to the center. For a further detailed knowledge on the solidification of the dispersed phase, the study on the solvent diffusion at the interface of the dispersed phase and the continuous phase is underway by using an interferometer technique disposed in the Université Libre de Bruxelles, Belgium.

Bibliography

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