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Effect of processing variables on the release and particulate properties of sustained release amoxicillin microcapsules prepared by emulsion solvent evaporation

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This study reports the preparation of amoxicillin microcapsules by an emulsion solvent evaporation process. In particular the effect of processing variables including the dimension and position of stirring paddle and container; volume of continuous phase versus dispersion phase; stirring speed and encapsulation temperature on the release and particulate properties of the amoxicillin microcapsules were determined. When the diameter of the paddle was half of that of the container and the clearance between the paddle and the bottom of the vessel was 1/4 of the total volume in the vessel, almost no material stuck to the inside wall of the beaker and uniform microcapsules were prepared. Very uniform, round microcapsules were also prepared with a high yield when $V_{acetone} : V_{light\ mineral\ oil} = 1:3$ and 1 : 5 because these systems ensured the formation of uniform emulsions. Physical evaluation of the microcapsules also showed that optimum drug release was achieved when the microcapsules were round, did not aggregate, were protected from the burst effect, the stirring speed for preparation was between 600–800 rpm and evaporation temperature was 25 °C. Microcapsules prepared using these ideal conditions achieved constant amoxicillin release for up to 12 h.

1. Introduction

Microencapsulation is a well-known method used to prepare controlled or sustained release dosage forms (Li et al. 1998). One method for the encapsulation of chemicals into water-insoluble polymers is the emulsion solvent-evaporation (ESE) process (Kim et al. 2002). This technique has been used successfully in the preparation of drug microspheres or capsules using different biocompatible polymers (Herrmann et al. 1998; Benoit et al. 1999; Das et al. 1998; Chen et al. 2000).

Many processing factors can influence the preparation of microcapsules by the ESE process. For example, the viscosity of polymer solution phase and molecular weight of polymers influenced the dissolution of theophylline microspheres prepared by the emulsion solvent-evaporation method (Obeidat et al. 2003). For salbutamol sulphate microspheres it was shown that the stirring speed influenced the release properties (Amperiadou et al. 1995). Palmieri et al. (2000) showed that the properties of paracetamol/polymer pellets prepared with different ratio of active ingredient and polymers were different. In this study, the addition of non-solvent prior to microencapulation was found to increase yield and the size of microspheres. In another study it was found that the addition of non-solvent also markedly changed the surface characteristics of the microsphere. Non-solvent produced coarser surfaces and an increased release rate (Yang et al. 2001).

However, there are few reports that comprehensively describe the effect of combinations of processing variables on the properties of microspheres or capsules prepared by the ESE process. The present study reports the effect of processing variables including, the dimension and position of stirring paddle and container; volume of continuous phase versus dispersion phase; stirring speed and encapsulation temperature on the release and particulate properties of sustained release amoxicillin microcapsules prepared by the ESE process.

2. Investigations, results and discussion

Paddles with diameters of 2, 4, and 6 cm and a beaker with a diameter of 8 cm were used to prepare the amoxicillin trihydrate (AMX) microcapsules while keeping other variables constant ($V_{acetone} : V_{light\ mineral\ oil} = 1:5$, concentration of emulsifier = 3%, concentration of ethylcellulose (EC) = 5%, W_{AMX}: $W_{EC} = 2:1$, bottom clearance = ¼ of the height of the system). It was found that when the diameter of the beaker was 4 times that of the paddle, a large percentage of the material stuck to the wall of the beaker, the microcapsules were not uniform in size and the microcapsules tend to aggregate. The reason for this was that the small paddle, compared to the container size, did not adequately stir the liquid phase to form a uniform emulsion and that the reduced stirring also increased the chance of emulsion droplets coalescing.

When the diameter of the paddle was increased to half of that of the container, almost no material stuck to the inside wall of the beaker and uniform microcapsules were prepared. In this case, a stable vortex formed while stirring indicating that a suitable stirring strength was applied to the whole system. This meant that the emulsion droplets solidified into microcapsules without contacting and sticking to each other. With a further increase in paddle size to a ratio of diameter of container to paddle of 4 : 3, most encapsulated material was forced against the wall of the container due to the high rotation force and the microcapsules were irregular shaped and not uniform in size. Based on the results of this experiment for the rest of the study reported in this paper, a dimension ratio between paddle and container of 1 : 2 was used.

The clearance between the paddle and the bottom of the vessel also influenced the microencapsulation process. This effect was studied by preparing microcapsules while varying the bottom clearances by 1/6, 1/4, 1/3 and 1/2 of the volume of the material in the mixing vessel. All other variables were kept constant, i.e., $V_{acetone} : V_{light\ mineral\ oil} = 1 : 5$, concentration of emulsifier = 3%, concentration of EC = 5%, W_{AMX}: $W_{EC} = 2 : 1$. The results are listed in Table 1. A clearance of 1/4–1/3 of the height of volume produced microcapsules with satisfactory morphology and yield. Based on these results a bottom clearance of 1/4 was used for the rest of the study.

Table 1: Effect of bottom clearance on microencapsulation by the ESE process

Bottom clearance	Observations	Yield (%)
1/6	Almost 1/2 the materials stuck to the wall of vessel; rod and spherical shaped microcapsules and agglomerates were formed	54.1
1/4	Little material stuck to mixing vessel; uniform spherical microcapsules were formed	92.7
1/3	Little material stuck to mixing vessel; uniform spherical microcapsules were formed	94.2
1/2	Almost a 1/3 of material stuck to the bottom of the beaker; uniform microcapsules and big aggregates were formed	65.3

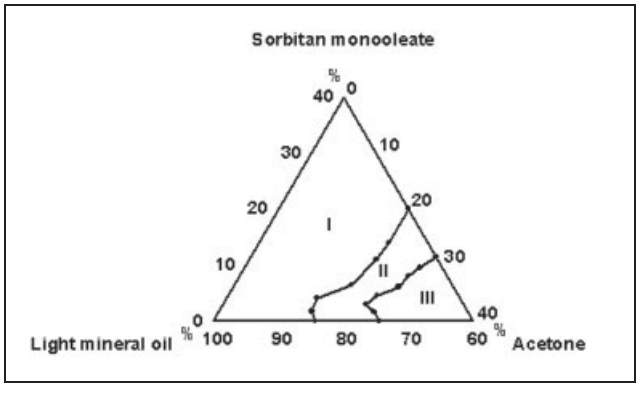

Fig. 1: Solubility phase diagram of light mineral oil – acetone – sorbitan monooleate: I, miscible region; II, emulsion region; III, immiscible region

To determine suitable ratios of acetone, light mineral oil and sorbitan monooleate, acetone was added to a series of mixtures of light mineral oil and sorbitan monooleate. The mixtures were shaken and then left to settle for 6 h before the separation into layers or the formation of emulsions were recorded. The results were used to construct a phase diagram as shown in Fig. 1. Using the same ratios used to construct the phase diagram shown in the Figure, microcapsules were prepared while keeping all the other variables the same (concentration of emulsifier = 3%, concentration of EC = 5%, $W_{AMX} : W_{EC} = 2 : 1$). Table 2 lists the morphological, particulate properties, and yield.

Very uniform, round microcapsules were prepared with a high yield when $V_{acetone} : V_{light\ mineral\ oil} = 1 : 3$ and $1 : 5$. According to the phase diagram, systems of $V_{acetone} : V_{light\ mineral\ oil} = 1 : 3$ and $1 : 5$ belong to region II, the emulsion region. Using these ratios, uniform emulsions formed and the emulsion drops solidified into round uniform microcapsules upon the complete evaporation of acetone. The microcapsules prepared with equal volumes of dispersion phase and continuous phase were not uniform in both shape and size because this ratio did not fall within the emulsion region in the phase diagram. When the volume of light mineral oil was increased to eight times that of acetone, the acetone evaporated so quickly that the microcapsule forming ingredients were solidified before a homogeneous emulsion was formed.

The drug release profiles from the microcapsules prepared with the different volumes of continuous phase and dispersion phase are shown in Fig. 2. It was found that the release of AMX from microcapsules prepared with $V_{acetone} : V_{light\ mineral\ oil}$ ratios of $1 : 3$ and $1 : 5$ was more consistent and repeatable than from those prepared with $V_{acetone} : V_{light\ mineral\ oil} = 1 : 1$ and $1 : 8$. Microcapsules prepared with the $1 : 1$ and $1 : 8$ rations displayed the so-called burst release effect (Huang and Brazel 2001). The least amount of AMX was released from microcapsules prepared with a ratio of $V_{acetone} : V_{light\ mineral\ oil} = 1 : 8$ because most of these microcapsules were aggregated. These

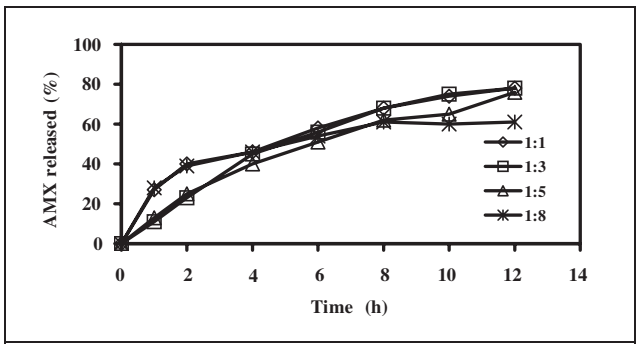

Fig. 2: Release profiles of amoxicillin from microcapsules prepared with different $V_{acetone} : V_{light\ mineral\ oil}$ ratios

Table 2: Effect of ratio of volume of continuous phase to that of the dispersion phase on the properties of the AMX microcapsules (n = 3)

$V_{acetone}$: $V_{light\ mineral\ oil}$	Morphology of MC	Roundness (ϕ, °)	Angle of repose (α, °)	Yield (%)
1 : 1	Rod-like, non-spherical; Powdered MC	27.8	42.3	78.0
1 : 3	Uniform spheres	15.4	28.0	94.5
1 : 5	Mostly uniform spheres	17.3	25.2	90.3
1 : 8	Agglomerates; irregular shaped MC	34.5	48.1	72.4

Table 3: Effect of stirring speed on the quality of microcapsules

Stirring Speed (rpm)	Morphology of MC	D_{50} (μm)	SPAN	Roundness (ϕ, °)	Angle of repose (α, °)	Yield (%)
600	Large spherical MC, some rod shaped	508	0.847	23.4	29.0	84.0
800	Uniform spherical	470	0.612	17.1	22.8	92.3
1000	Uniform spherical	431	0.538	15.4	20.0	94.7
1200	Small spherical MC with powder	365	0.912	18.3	24.6	90.5

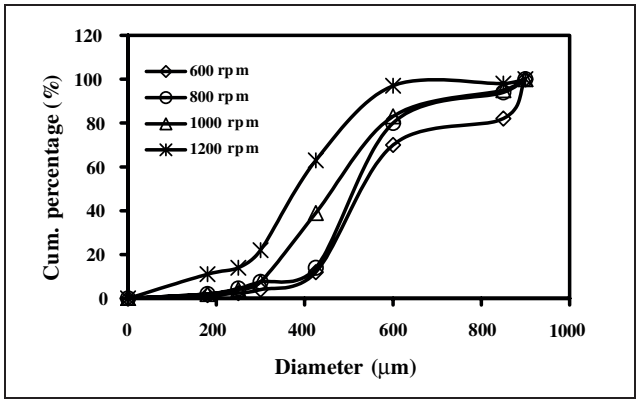

Fig. 3: Effect of stirring speed on the size distribution of AMX microcapsules

results implied that drug release from the microcapsules was influenced by the roundness of the microcapsules, the degree of aggregation and the burst effect. The control of drug release was better from rounder, individual microcapsules. Therefore, a $V_{acetone} : V_{light\ mineral\ oil}$ ratio of 1 : 5 was used for the rest of the study.

The effect of stirring speed on microencapsulation and the morphology, particle size, yield and release of AMX from the microcapsule was studied by preparing microcapsules at different stirring speeds, 600, 800, 1000, and 1200 rpm when all other experimental conditions were the same (concentration of emulsifier = 3%, concentration of EC = 5%, $W_{AMX} : W_{EC} = 2 : 1$). The properties of the microcapsules prepared at these speeds are listed in Table 3. The difference in size distribution of AMX microcapsules prepared at the different stirring speeds are shown in Fig. 3. Table 3 and Fig. 3 show that the mean microcapsule size (D_{50}) was reduced as the stirring speed was increased from 600 to 1200 rpm. This was because the emulsion of AMX-EC-acetone formed smaller droplets under high shear and these smaller droplets of emulsion formed microcapsule with smaller diameters. The AMX microcapsules therefore had a narrower particle size distribution when the stirring speed was between 600 and 800 rpm. As shown in the Table a change in stirring speed had minor impacts on the roundness and flowability of the microcapsules.

The release profiles of AMX from the microcapsules with a change in stirring speed are shown in Fig. 4. The release rate of AMX from the microcapsules increased with an increase of stirring speed because the particle size of the microcapsules decreased with an increase in stirring speed. This means surface area for release available per weight of microcapsules was increased. The release of AMX was best when the stirring speed was between 600 to 800 rpm. The release of AMX was too fast when the stirring speed was 1200 rpm.

Microencapsulation temperature is a key variable in the encapsulation and solidification process during an emulsion solvent evaporation process. The effect of evaporation temperature on the quality of the microcapsules was studied by encapsulating AMX at different temperatures, namely, 15, 25, and 40 °C with concentration of emulsifier = 3%, concentration of EC = 5%, $W_{AMX} : W_{EC} = 2 : 1$, and a stirring speed of 600 rpm. The results are shown in Table 4 and Fig. 5 and 6.

A change in microencapsulation temperature influenced the yield, evaporation time needed, and the properties of MC. The results shown in Table 4 and Fig. 5 and 6 showed that the temperature should not be too low because at 15 °C the acetone evaporated too slowly allowing for the formation of very small liquid drops that produced small microcapsules. Furthermore, as the evaporation time needed for all solvent to evaporate increased, the chances for the liquid drops to hit each other also increased, which increased the aggregation of the microcapsules. When the evaporation temperature was 40 °C, solvent evaporation was too fast to allow the formation of a uniform emulsion. This meant that the microcapsules were not uniform in size and the bulk density of the microcapsules was less due to high surface porosity introduced by the fast solvent evaporation. Very uniform microcapsules were prepared at 25 °C.

The yield was not influenced significantly by temperature, however, somewhat more raw material attached on the surface of the container at 40 °C, which reduced the yield. The reason for this was that acetone evaporated too fast

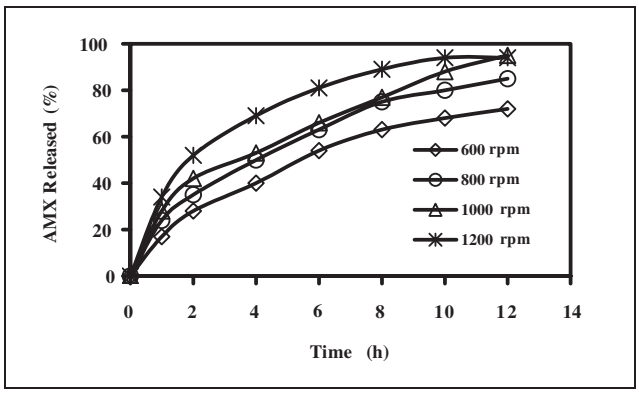

Fig. 4: Release profiles of AMX from microcapsules prepared at different stirring speeds

Table 4: The effect of evaporation rate on the properties of the AMX microcapsules

Temperature (°C)	Morphology	Roundness (ϕ, °)	Angle of Repose (α, °)	Tapped density (g/ml)	Yield (%)	Evaporation time (h)
15	Fine MC and aggregates	32.5	35.4	0.52	92.0	4.5
25	Uniform MC	19.6	26.3	0.48	93.8	3.0
40	Non-uniform MC	30.0	34.8	0.40	87.0	1.0

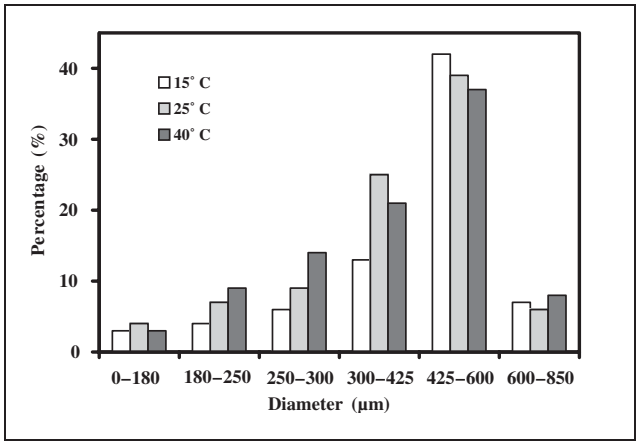

Fig. 5: Effect of evaporation temperature on the size distribution of AMX microcapsules

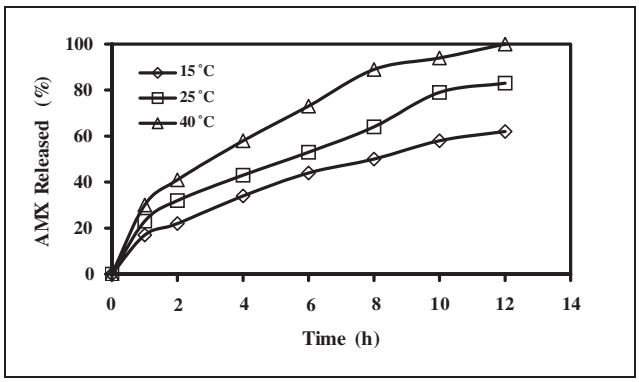

Fig. 6: Release profiles of amoxicillin from microcapsules prepared using different evaporation temperatures

from the surface of the emulsion drops that were close to the side of the container, which allowed the emulsion drops to solidify against the wall.

The results in Fig. 5 show that the evaporation temperature influenced the release rate of AMX. At higher evaporation temperatures, looser EC matrixes with larger surface pores were formed. Faster penetration of the dissolution medium into these capsules increased the release of AMX. The best and most consistent release profiles, Fig. 6, were obtained from microcapsules prepared at lower temperatures.

In conclusion, the size and dimension of the mixing vessel and position and size of the stirring paddle, along with the ratio of continuous phase and dispersion phase, stirring speed and microencapsulation temperature, are the main processing variables that determine the successful preparation of sustained release amoxicillin microcapsules by an emulsion solvent evaporation process. These variables not only influenced the morphology and particulate properties of the microcapsules but also determined the release of the drug from the microcapsules.

3. Experimental

3.1. Materials

Amoxicillin (Lot. No. 1998101102) was purchased from Hebei Pharmaceutical Company (Hebei, China). Ethylcellulose (EC) was kindly donated by Shanghai Kalekang Company (Shanghai, China). Acetone (A.C.S. grade) was obtained from Shenyang Chemical Company (Liaoning, China). Light mineral oil was purchased from Kaiyuan Chemical Manufacturing Company (Jilin, China). Sorbitan monooleate was a product of Dalian Chemical Company (Liaoning, China).

3.2. Preparation of microcapsules

The oil-in-oil emulsion method was applied to prepare amoxicillin microcapsules (AMX-MC). A suspension of amoxicillin in a solution of EC in acetone was added to a mixture of sorbitan monooleate and light mineral oil. The system was stirred with an electronic-craft stirrer (Shanghai Electrocraft Corporation, China) at constant speed until the evaporation of the acetone was complete. The microcapsules were collected by filtration, washed with hexane three times and dried in a vacuum oven at room temperature.

3.3. Evaluation of microcapsules

3.3.1. Encapsulation efficiency and yield

An accurately weighed amount of amoxicillin microcapsules was ground into uniform white suspension in the presence of a small amount of water. The white suspension was diluted with water and then ultra-sonicated for 10 min to ensure complete dissolution of amoxicillin into water. The solution was filtered to remove undissolved materials. The filtrate was diluted and assayed with the imidazole method (Chinese Pharmacopoeia 1995). The content of active ingredient in the microcapsules was calculated from a standard curve ($\lambda_{max} = 325$ nm; $A = 2.29 \times 10^{-3} + 5.70 \times 10^{-2}C$, $R = 0.999$, $n = 6$). The following two equations were used to calculate encapsulation efficiency and yield as described by Jiang et al (1993):

$$\text{Encapsulation efficacy (\%)} = \frac{\text{Weight of MC} \times \text{conc. AMX}}{\text{Weight of AMX used}} \times 100 \quad (1)$$

$$\text{Yield (\%)} = \frac{\text{Weight of } 500 - 850\,\mu\text{m MC}}{\text{Total weight of material}} \times 100 \quad (2)$$

3.3.2. Particulate properties

The characteristics of microcapsules that were measured included particle size, roundness, flowability, bulk density, and morphology. The sieve method was used to determine particle size. A group of sieves were stacked and approximately 1 g of microcapsules were placed on the top screen and vibrated mechanically with a shaker (AR400, Erweka, Germany) for 10 min. The amount of microcapsules on each screen was accurately weighed. The size distribution (polydispersity) was measured as in terms of a SPAN factor expressed as:

$$\text{SPAN} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (3)$$

Where $D_{90\%}$, $D_{10\%}$ and $D_{50\%}$ are the diameters where the given percentage of particles is smaller than that size. A high value of SPAN indicates a wide size distribution and a high polydispersity (Torrado et al. 1989).

The roundness of the microcapsules was estimated by the angle at which the microcapsules started to flow when placed on a piece of clean glass and one end of the glass was lifted. The smaller the angle of flow the rounder the MC's. The angle of repose (α) was measured using the funnel method with fixed base cone (Aulton 1988). The smaller the angle of repose, the better the flowability and the roundness of the MC's. The tapped density was measured by loading a 10 ml graded cylinder with 1 g of MC's. The cylinder was tapped by dropping it three times freely from a height of 5 cm onto a desktop. The measured volume was recorded and the bulk density calculated using the equation: $d = m/v$, where d is tapped density, m is weight of MC and v is volume of MC.

3.3.3 Amoxicillin release

The USP method II or paddle dissolution test was used with a pH 6.8 PBS as the release medium. The stirring speed was 100 rpm and the temperature of the release medium was kept at 37 ± 0.5 °C. Accurately weighed microcapsules containing about 287 mg AMX trihydrate which is equivalent to 250 mg anhydrous AMX was added into each dissolution flask. Five ml solution was taken from each of six flasks after 1, 2, 3, 4, 6, 8, 10 and 12 h. To replace the amount withdrawn at each time point 5 ml release medium preheated to 37 °C was added to each flask. The withdrawn solution was filtered and assayed by determining the UV absorption at 272 nm. The accumulated amount of AMX released from AMX-MC was calculated from the standard curve ($\lambda_{max} = 272$ nm; $A = 1.89 \times 10^{-3} + 2.60 \times 10^{-3}C$, $R = 0.999$, $n = 6$).

3.4. Statistical analysis

All statistical evaluations were performed using SAS 6.12 (SAS Institute Inc., Cary NC, USA).

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