Supercritical Antisolvent Processing of Insulin/HPMCP Nanocapsule Preparation

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Abstract: In order to develop new drug-delivery system of insulin, Hydroxypropylmethyl Cellulose Phthalate(HPMCP), a kind of biodegradable polymer with enteric solubility, was applied in preparation of nanocapsules which attracted more and more attention in pharmaceutical field. Nanonization of insulin loaded by HPMCP using continue supercritical antisolvent(SAS) processing was studied in this research. Pure CO_2 and DMSO/DCM were as an anti-solvent and solvent in SAS process respectively. A series of operating parameters，such as pressure, temperature, concentration of HPMCP and insulin, flow rate of CO_2 and insulin/HPMCP ratio in solution, were analyzed to optimize the process of preparation. It was found that the produced capsules were in nanoscale(about 200nm to 700nm), drug loading kept at 11% and encapsulation efficiency reached 75% .
Keyword: supercritical antisolvent insulin HPMCP nanocapsule

INTRODUNCTION

Insulin is the most common and efficient drug in diabetes therapy. It is known to all that patients have to take it for their whole life. The most common method of taking insulin is injection, but it is not convenient for patients. So a new drug-delivery system should be applied to help diabetes patients. Encapsulation of insulin is a good method in which drug could be protected from biodegradation while taking in oral, the release of rate could be controlled and the duration of bioactive protein could be prolonged [1]. Another advantage of encapsulation is that its tiny figure makes it spread into cell easily, especially infiltrate into capillary vessel.

A newly developed method, supercritical anti-solvent process (SAS), has been applied widely in preparing micro/nanocapsules because it can avoid using organic solvent excessively and work under gentle conditions[2-13]. In SAS process, solute was dissolved in solvent at first, then, supercritical CO_2 was sprayed into solution as an anti-solvent. Supercritical CO_2could induce phase separation where the solute precipitated due to a high supersaturation generated by the mutual diffusion of organic solvent into CO_2 and vice versa when an organic solution contacted with CO_2. Nanoparticles were obtained after being dried by pure CO_2 [14].

We applied Hydroxypropyl methyl cellulose phthalate (HPMCP), one kind of cellulose derivates, as coating material, which was confirmed as enteric carrier and was applied in drug industry widely from 1971 when it was on market. HPMCP could maintain good stability in acidic conditions such as in stomach and also could control the release rate of some drug in enteric condition, because it was a pH-sensitive polymer (It could be dissolved in the environment of pH greater than or equal to 5.5.) It was

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attributed by the structure of HPMCP as shown in Figure 1, the phthalyl substituents would influence the hydration and coacervate formation with the variety of pH value.

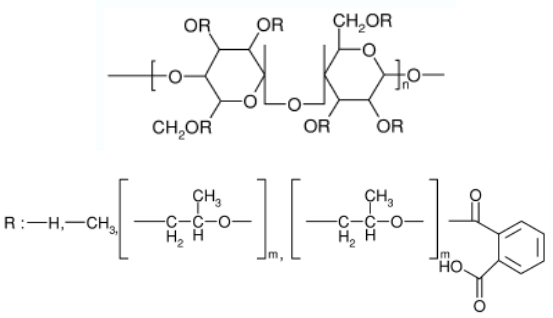


Figure 1 The molecular structure of HPMCP

In this research, some parameters such as pressure, temperature, concentration and the flow rate of CO_2 were studied and discussed how they influenced the SAS process and the nanocapsules.

MATERIALS AND METHODS

Material

Insulin, extracted from pig pancreas, was obtained from Wanbang biologic pharmacy Ltd(Jiangsu, China).

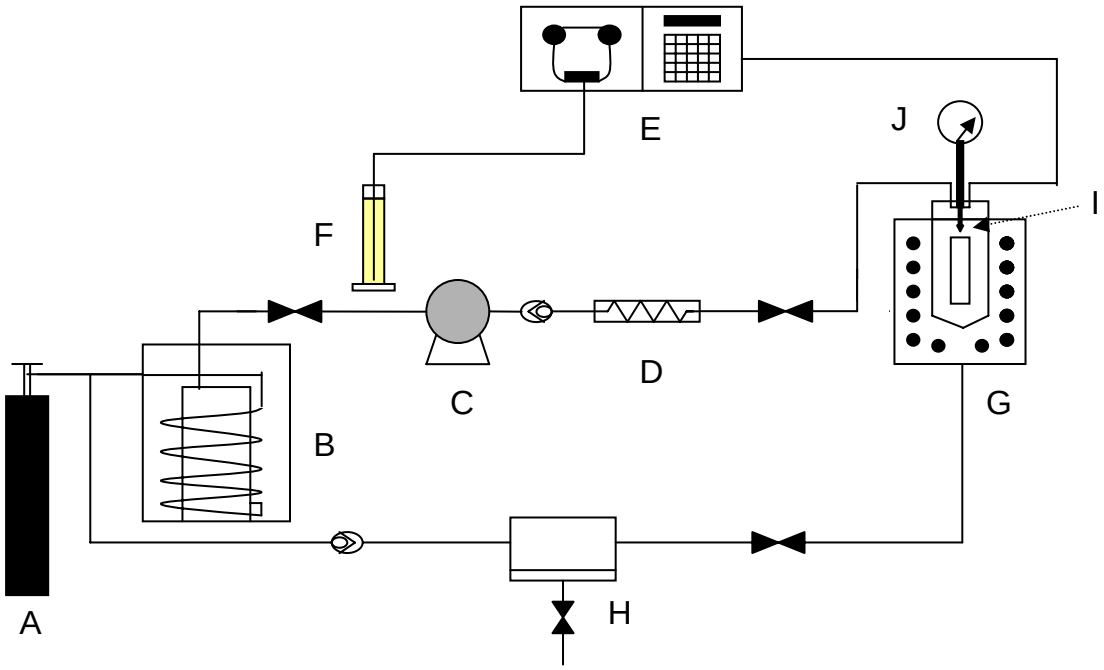
Hydroxypropyl methyl cellulose phthalate (HP55), ρ=1.28g/cm3,η=32-48 mPa·s, was supplied by Hopetop Ltd(Jiang, China).

DMSO(AR) and DCM(AR) was purchased from SCRC Ltd(Shanghai, China).

Preparation of insulin/HPMCP nanocapsules by SAS process

Insulin and HPMCP were dissolved into DMSO/DCM mixed solvent (1:1, volume ratio).

In SAS experiment, as shown in fig.2, CO_2 from the cylinder (A) was firstly cooled to form liquid by refrigerator (cooling system). And CO_2 was pumped into the precipitating vessel (G) by the piston pump(C) via the heat exchanger (D) and the nozzle(I). CO_2 then went into the separator from the precipitating vessel and back to the refrigerator for circulation. After the pressure and temperature reached the presetting values, the liquid solution was injected though the nozzle and premixed with SC-CO_2 before entering the vessel. The flow of liquid solution could be adjusted by an HPLC pump (E). When all the solution finished to be injected in the vessel, SCF CO_2 continued to be pumped into the vessel (G) for some time in order to remove the solvent to make the products with no solvent remaining. The organic solvent could be separated in the separator (H) from the CO_2 and for circulation use.



A-CO_2 cylinder B-cooling system(refrigerator) C-piston pump D- heat exchanger E-HPLC pump F-solution G-vessel(precipitator) H-separator I-nozzle J-pressure meter

Figure 2 Scheme of the apparatus of SAS experiment

Morphology observation

Morphology characterizations of nanoparticles were observed by TEM (JEOL, JEM-2010) and SEM (JOEL, JEM-7401F). TEM was applied to observe the inner morphology of the nanoparticles. The sample was dispersed in ethanol by ultrasonic, and then dried in the room temperature. In SEM observation, the specimens were coated with sputtered palladium for 20s to make the surface conductive without compromising fine surface microstructure. A nonconductive surface would produce a severe surface charge problem under the high intensity electron beam and accumulated surface charge would cause abnormal contrast, image deformation and distortion.

Particle size distribution

Particle size distribution of insulin/HPMCP nanocapsules were characterized by a particle size analyzer (ZETA SIZER, nano series, Malvern). 2mg nanocapsules were put into 5ml pure water, dispersed equably by ultrasonic and then diluted to 20ml by pure water. The test temperature was 25℃. We should analyze each sample for 3 times and obtain the average value as results.

Drug loading and encapsulation efficiency

According to Chinese Pharmacopoeia, the standard curve of insulin was made. 0.01mol/ml HCl aqueous solution was applied to quantitative analysis.

To weigh 10mg nanocapsules exactly, mix them with 10ml 0.01mol/ml HCl aqueous solution for 5 minutes, then filter and take 2ml to test the concentration of insulin by UV spectrum (SP-732, Spectrum Ltd, Shanghai, China) and evaluate the amount of insulin uncoated in HPMCP. Next step was to calculate total amount of insulin in each sample. To make up the mixture as before, shake it by ultrasonic, filter 2ml to test the concentration of insulin by UV for several times until the concentration kept in a fixed value. The Drug loading and Encapsulation Efficiency were calculated by following equation (1) and (2):

Drug Loading (DL)= M_{ip} /M_p×100% (1)

Encapsulation Efficiency (EE)= $[1-M_{ui}/M_{ip}]\times 100\%$ (2)

M_{ip}: Mass of insulin in the product; M_p: Mass of product; M_{ui}: Mass of uncoated insulin

RESULTS AND DISCUSSIONS

Table 1 SAS experimental conditions and results

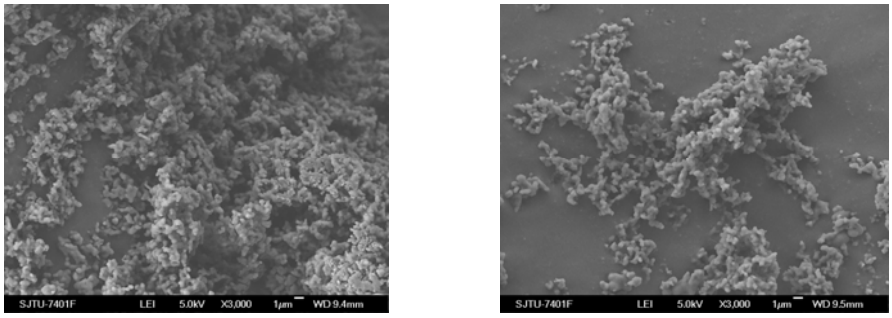
No	c_{HPMCP} (mg/ml)	$C_{insulin}$ (mg/ml)	$v_{solution}$ (ml/min)	v_{CO2}(kg/h)	T (℃)	P (MPa)	DL(%)	EE(%)	d_m (nm)
1	1	0.1	1	1.50	32	15	11.53	75.20	306
2	1	0.1	1	1.50	35	15	7.11	55.64	515
3	1	0.1	1	1.50	38	15	6.51	49.72	769
4	1	0.1	1	1.50	35	9	5.38	49.55	255
5	1	0.1	1	1.50	35	12	6.27	62.04	336
6	1	0.1	1	2.00	38	15	4.48	55.18	451
7	1	0.2	1	1.50	35	12	5.19	35.47	273

The effect of processing parameters

According to the No.1, 2 and 3 of Table 1, the DL and EE decreased with increasing of temperature. Under the condition of 32℃/15MPa, the DL value was 11.53% which was higher than the initial ratio of insulin(9.09%) and the EE value reach at 75.20%. That indicated the precipitating rate of insulin was larger than that of HPMCP under such condition. The mean diameters of products were shown in Table 1, the particle size became larger at higher temperature. Camparing with experiment No.4, 5 and 2, the pressure had a bit effect on DL value and EE from 9MPa to 15MPa. The mean diameter of nanocapsules increased with pressure increasing. In experiment No.3 and 6, the result indicated that higher flow rate of CO_2 decreased DL, because the variety of the proportion among CO_2, mixed solvent and solute(insulin and HPMCP) could influence the solubility of solute in the solvent and the process of precipitation. To evaluate effect of the ratio of insulin and HPMCP in solution on DL and EE, according to the result of experiment No.5 and 7, only increasing the amount of insulin could not make the DL and EE higher, even reduced these two values. Hence the ratio between drug and coating material should be controlled suitably.

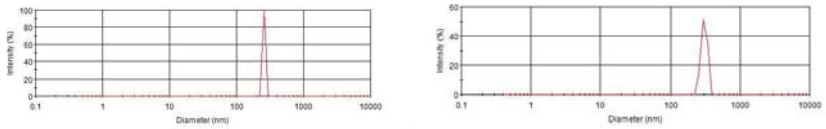
Morphology observation

The SEM photos of capsules which were prepared under 38℃/15MPa and 35℃/12MPa, were shown in Figure 3, they indicated that most of nanocapsules were sphere or elliptical shape and well-proportioned, but the aggregation of particles was found. In Figure 4, PSD analyzing of samples(35℃/9MPa and 32℃/15MPa) shows that the nanocapsules had a narrow size distribution. The sample prepared under 35℃/9MPa was observed by TEM(Figure 5), the dark zone which distributed in the light sphere was conducted by XRD. The result indicted that most of insulin was encapsulated in HPMCP, because HPMCP was a kind of amorphous polymer and insulin was protein crystal. It should be paid attention to that the dispersant in TEM and PSD analyze was different, so the measured particle size was various. In ethanol system, the dispersal effect seemed better than in water system, so in TEM photo we could observe smaller particles under the same conditions.



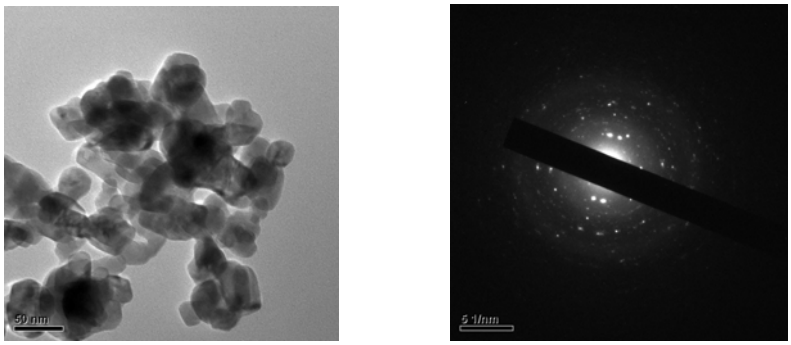
A 38℃/15MPa B 35℃/12MPa

Figure 3 The SEM photos of insulin/HPMCP nanocapsules



A 35℃/9MPa B 32℃/15MPa

Figure 4 PSD of insulin/HPMCP nanocapsules



A 35℃/9MPa B XRD

Figure 5 The TEM photo and XRD of insulin/HPMCP nanocapsules

CONCLUSION

In this research, insulin was encapsulated by HPMCP using continue supercritical antisolvent(SAS) processing. A series of operating parameters were analyzed to optimize the process of preparation. Temperature, flow rate of CO_2 and the ratio of insulin and HPMCP had obvious effects on DL and EE. Temperature and pressure could influence PS of nanocapsules. Moreover, to control the ratio of insulin and HPMCP was also a key way to increase the DL and EE of nanocapsules. Finally, it was found that the produced capsules were all in nanoscale(about 200nm to 700nm). Drug loading and encapsulation efficiency could reach 11% and 75%, respectively.

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