Nanoencapsulation of Insulin with HPMCP Using Supercritical Antisolvent Technique

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INTRODUCTION

It is estimated that the number of diabetic patients will reach to 366 million in 2030. Insulin is common known to be the most efficient to diabetes and remains the main treatment for Type I and Type II diabetic patients. Its routine delivery is through daily subcutaneous injections, which would give a negative quality of life of diabetic patients, especially for patients who need taking insulin for life. So the other alternative routes of administering insulin such as oral and nasal pathways have been investigating for the years [Lin Y. et al. 2007]. Oral delivery of insulin is the most comfortable and welcome. However, insulin is poor absorbed by the intestinal mucosa and is rapidly degraded enzymatically in the gastrointestinal tract. Then, it is highly demanded to develop new formulation strategies to maintain the stability and increase the bioavailability of insulin by assembling insulin and excipients into a physical structure. The nanoparticles made with biodegradable and biocompatible polymer with insulin have been developed. These nanoparticles can protect insulin against degradation and facilitate uptaking [Malam A. et al. 2000, Anne D.et al. 2006]. Some traditional processing techniques, such as interface aggregation, milling and spray drying et al, have been used in preparation of microcapsules. But these processes either involve large organic solvent, high temperature or complicated steps.

In order to overcome the drawbacks mentioned above, supercritical carbon dioxide antisolvent technique (SAS) is developed [Chang S. et al. 2008]. In SAS process solute is dissolved in solvent firstly then, SCF mixed with solution through a nozzle spraying. The solute can precipitate very quickly with diameter small and uniform distribution due to a high supersaturation degree generated by the mutual diffusion of organic solvent and SCF. William K. et al. (2002) obtained insulin powder by PCA. Nicola E. et al. (2001) produced insulin-loaded PLA by SAS. But nanometer scale of insulin encapsulated with biodegradable polymer has not gotten yet.

In this paper process variables, such as pressure, temperature, the ratio of mixed solvent, CO_2 flow rate, solution flow rate, concentration of solution, et al, are investigated in detail in order to make insulin-load particle with Hydroxypropylmethyl Cellulose phthalate (HPMCP) using SAS. The particles are characterized by SEM in terms of particle morphology, particle size, size distribution, insulin loading and encapsulation efficiency. Only part of the experimental results is given here because of the space limit of the paper.

MATERIALS AND METHODS

DMSO (AR), acetone (AR) and PBS (PH 7.4 and PH 1.4) are purchased from SCRC, China, HPMCP (Hydroxypropylmethyl Cellulose phthalate, HP55) is supplied with Hopetop Ltd. (Jiangsu, China). Insulin obtained from Jiangsu Wanbang biologic pharmacy company. CO_2 with the purity of 99.95% obtained from SJTU Gas Station (Shanghai, China).

Preparing nanocapsules by SAS process

In SAS experiment, as shown in Figure 2, CO_2 from the cylinder (A) is firstly cooled to form liquid by the chilling system (B). And CO_2 is pumped into the precipitating precipitation vessel (G, V=200cm^3) by the high pressure pump (C) via the heat exchanger (D) and the nozzle (I). CO_2 then goes into the separator from the precipitation vessel and back to the B for circulation. After the pressure and temperature reached the presetting values, the liquid solution is injected though the nozzle and premixed with SC- CO_2 before entering the vessel. Figure 3 shows the inner structure of nozzle. The nozzle consists of two parts, like a bushing, the solution sprayed into the vessel through the inner tubule (Φ=0.2mm) and CO_2 spayed into G through the outside part (Φ=1mm). The inner part is shorter than the outside. This structure can make the solution mix with CO_2 more efficiently, and get better atomization of solution. The solvent power of the original solvent in the solution would reduce along with high diffusion of SCF CO_2 in the solution and then the solution can reach rapidly to the high supersaturation state, the solute would precipitate in the vessel and the solvent would be taken by SCF CO_2. This process can be also illustrated in Figure 3. The vessel has two glass windows through which the phenomena, such as precipitation could be observed. After all the solution is injected into the vessel, SCF CO_2 continues to be pumped into the vessel (G) for some time in order to remove the solvent. The organic solvent can be separated in the separator (H) from the CO_2 and for circulation use. Finally, the pressure of the vessel is adjusted to the atmosphere and precipitated nanoparticles are taken out and characterized.

Figure1 Scheme of the apparatus of SAS A-CO_2 cylinder, B-chilling, C-pump, D-heat exchanger, E-HPLC pump, F-solution, G-vessel,H-separator,I-nozzle,J-pressure meter

Figure2 The structure of the nuzzle and the illustration of formation of nanocapsules

Morphology

SEM (JOEL, JEM-7401F) is used to observe the morphology of samples. TEM is applied to observe the inner morphology of the nanoparticles.

Particle size distribution (PSD)

Particle size distribution of samples is characterized by a particle size analyzer (ZETA SIZER, nano series, Malvern).

Insulin loading (II)

The drug loading is defined as the ratio of amount of insulin in the sample and the sample. Weigh an amount of sample accurately and dissolve them in PBS at PH 7.4. UV spectrometer is used to measure the absorbance. The content of insulin in the sample is calculated from the standard curve.

Encapsulation efficiency (EE)

EE is defined as the ratio of the amount of insulin encapsulated in HPMCP and the total amount of insulin in the sample. Weigh an amount of samples accurately and dissolve them in PBS at PH 1.4. UV spectrometer is used to determine the absorbance.The insulin content is calculated from the standard curve, which is taken as the insulin content on the surface of the sample as HPMCP could not be dissolved in acid PBS. EE is gotten by deducting the insulin amount on the surface from the total insulin amount in the sample obtained as in characterization 3.

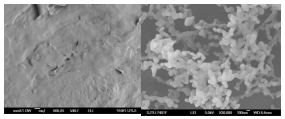
RESULTS AND DISCUSSION

Preparation of HPMCP nanoparticles using SAS

The experimental conditions and results are listed in Table 1. Beside, the solution flow rate is 1mg/ml but No.7 is 2mg/ml; CO_2 flow rate is 1.5kg/hr, but No6 is 3kg/hr, concentration is 3.33mg/ml but No8 is 1mg/ml. It can be seen from Table 1 that the 170nm of HPMCP particle is obtained. The particle size is smaller when CO2 flow rate is higher shown in No6. Solution flow rate has little influence on particle size. The lower temperature is, the smaller the particle like No4. Pressure has impact on particle size as shown in No1-3. Fig.1. show the influence of solvent type, such as DMSO and Acetone, on the morphology of HPMCP processed by SAS. It can be seen that there exists a big difference between DMSO and Acetone. Nanoparticles of HPMCP could be obtained when acetone is used as a solvent but not for DMSO. Then, the type of solvent would have impact on the preparation of nanoparticle using SAS.

N°	T (℃)	P (MPa)	Solvent Ratio (DMSO/Atetone)	Particle mean diameter
1	40	8	1/3	------
2	40	16	1/3	392
3	40	12	1/3	448
4	32	12	1/3	260
5	32	12	1/5	240
6	32	12	1/5	226
7	32	12	1/5	244
8	40	12	0/1	170

Table 1. Experimental conditions and results of HPMCP processed by SAS



A B

Fig.1 SEM of HPMCP particle (A:DMSO ; B:Acetone)

Nanoencapsulation of insulin with HPMCP

On the basis of preparation of HPMCP nanoparticles, nanoencapsulation of insulin with HPMCP using SAS techniques is investigated. In this case only the ratio between the amount of insulin and HPMCP varies. Other variables do not change. The temperature is 32℃, the pressure is 12MPa, the solvent ratio is 1/5(DMSO/Acetone), the insulin concentration is 0.33mg/ml, the CO_2 flow rate is 5kg/h and the solution flow rate is 1ml/min. The experimental conditions and results are shown in Table 2. It is clear that insulin can be loaded in HPMCP with size ranging from 342nm to 138nm. The lower HPMCP concentration is, the smaller the particles. We can also see the amount of insulin loading is different from that of the initial made loading from which it is inferred that the precipitate amount of insulin is different from that of HPMCP during SAS process. And the amount of insulin loading can increase a little when the ratio between the insulin and HPMCP increases. But it does not always increase along with the increase of the insulin initial amount. The reason might be that the precipitate amount of HPMCP is not enough to encapsulate insulin when its initial amount is too low Then it is important to choose suitable concentration of coating materials. And it is also necessary to choose the suitable amount ratio of loaded material and coating material. Moreover, all the encapsulation efficiency of these experiments is above 99.0%. That means nearly all of insulin is encapsulated in HPMCP particles. The reason might be that insulin is almost indeed embedded into the HPMCP or all the insulin on the surface of HPMCP particles is removed by SC-CO_2 during the washing process. Fig.2. shows clearly that insulin is encapsulated in HPMCP whose size is about 100nm. Release of insulin-loaded nanoparticle of HPMCP is also studied in PBS solution.

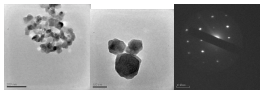


Fig.2 TEM image of insulin encapsulated in HPMCP particle

No	HPMCP concentration (mg/ml)	Insulin initial loading%	Insulin loading %	Mean Particle diameter (nm)	EE%
1	6.67	4.7	10.76	342	99.3
2	3.33	9.0	16.04	265	99.1
3	1.67	16.5	14.46	138	98.5

Table 2. Experimental conditions and results of encapsulation of insulin with HPMCP

CONCLUSIONS

The nanoencapsulation of insulin with HPMCP is successfully prepared using SAS technique. Insulin loading reachs the range from 10.8% to 16.0%. Encapsulation efficiency is more than 98.5%.

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