Effect of nozzle structure on the size and morphology of zein nanoparticles obtained by SEDS process

Sining Li; Wen Shi; Daode Hu;Yaping Zhao[a]

[a] (School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China)

Abstract: Preparation of zein nanoparticles using SEDS (solution enhanced dispersion by supercritical fluid) process has been published. An optimized process conditions (ie., temperature,pressure,solutions) had been determined based on the previous work. The effect of nozzle structure on the size and morphology of zein nanoparticles was studied through an new designed nozzle which the relative position of the two tubes in coaxial nozzle could be changed. In three different structure we obtained very different particles, Spherical particles which diameter is 100-300nm was obtained in convex condition that is similar with the previous work, however, in level condition a filamentous particles was obtained, the thread diameter was about 20-40nm. In concave condition, the morphology of obtained particles was between the two cases above. The flow rate of supercritical CO_2 was also studied as variable, but the effect was not as significant as the changes of nozzle structure. An simplified model was established for simulation. Flow and concentration field distribution was calculate by CFD(computational fluid dynamics) software to explain such a big difference on the morphology of the particles in different nozzle structures.

Key words: zein nanoparticles; nozzle structure; filamentous; CFD

Introduction

The unique properties of fine particles have been aroused great interest for decades. Several manufacturing process had been developed to obtain microsized and nanosized particles such as lyophilization [1], spray drying [2]and supercritical method etc. Compared with other methords, supercritical antisolvent (SAS) technology which has unique advantages for not only the specific characteristics of fluids at supercritical conditions, like the adjustable solvent power and the gas-like diffusivity but also its flexibility in operating and applicability in materials is one of the hotspot recently. In addition to the traditional way, SAS is also known as other appellation based on different injection device, like gas antisolvent (GAS), aerosol solvent extraction systems (ASES) particles from gas-saturated solutions (PGSS) , supercritical antisolvent with enhanced mass transfer (SAS-EM) , and solution-enhanced dispersion by supercritical fluids (SEDS)[3-7].

The SEDS process is a modified SAS process in order to achieve smaller droplet size and enhance the mass transfer of SF and the solution by larger interface and faster surface renewable. The supercritical fluid is used both as anti-solvent and as a ‘spray enhancer' by hydro mechanical effects[8,9]. The core unit is a special coaxial nozzle via which the supercritical fluid and the liquid solution are injected into a high-pressure vessel simultaneously. Currently, many kinds of particles were prepared through this process from single component particles such as lactic acid-based polymers (l-PLA, d,l-PLA), puerarin, and β-carotene [10-13] to multi-component composite particles such as PLGA loaded with lysozyme[14], PHBV loaded with β-carotene[13]. The size of most of these particles was between 100-500nm.

However this process was so complex for it contained lots of adjustable parameters which can affect the results[15] that the understanding of the process is far from completion. These parameters can be divided into two categories, thermodynamic factors and kinetic factors. Temperature, pressure and an organic solvent are the mostly studied thermodynamic factors. They are the key factors determining the phase equilibrium state of the system which affect formation of the particle so as the size and morphology of the particle. Although they have different trend on particle formation in different material system, there is a consensus that most of the particles which meet the requirements are obtained above the critical point of the mixed solvent[16-18]. Meanwhile the kinetic factors which affect fluid flow and mass transfer such as the flow rate of solvent and supercritical CO_2, injection from, and nozzle structure et al.. also affect the size and morphology of the particle obviously.

Investigation of the flow rate appeared in most previous studies. Thus most of them speculate the impact of the flow process only through the difference in particles since it is hard to study flow field directly. In recent days, calculating flow field becomes realistic with the maturity of computational fluid dynamics(CFD) technology. And M.A. Tavares Cardoso got good results by combined SAS with CFD[19].

The impact of nozzle structure which is a key factor especially in SEDS process on the particle formation process should not be ignored. It can be expected that the nozzle structure determine the mixed way of the two flows thus exert a decisive influence on the mass transfer between the two phases. However such research was mentioned rarely except few researchers like Andre anne Bouchard [20]. In this article, a newly designed coaxial nozzle which has a adjustable inner pipe was uesd to study the impact of diffenent nozzle structure and for further investigation the CFD technology was applied to SEDS process based on previous research.

Maize zein is the main storage proteins of maize seeds and account for 50% or more of total endospermic proteins. The zein proteins are hydrophobic and insoluble in pure ethanol and thus it could dissolved in ethanol mixed with many other solvents such as water, acetone and dichloromethane etc. Zein has long been of both scientific interest and industrial importance. As a material with good biocompatibility , it has been widely utilized in drug release system such as 5-fluorouracil-loaded zein nanoparticles[21] lysozyme-loaded zein microcapsules [22] etc.. And another application is carrier in release system in the field of food [10,22]

There are many ways to obtain zein particles include evaporation[16], spray dry[21], liquid–liquid dispersion[10] and , certainly the SAS process wihch produced a wide range of nano-sized particle is also proved to be a good way[22]. Yunchang Guo reports that there are two types of particles, globular and rod-like. In some conditions, zein could form fiber-like structure. It is sure that zein paticles in different size and shape could be used in different direction such as coating material in pharmaceutical or biodegradable material in package. Due to the diversity of shapes zein is used as model compounds in this paper.

The main purposed of this research was to explore how the nozzle structure affect the formation of particles. So the newly designed nozzle which included adjustable structure was used to obtain nano-sized zein particle. Different structure of nozzle and $SCCO_2$ flow rate was investigated by compare the size and shape of nano-sized zein particle. In addition, a CFD model was established to calculate the flow field to give a general explanation of the difference in products.

2. Materials and methods

2. Materials and methods2.1. Materials

Zein was purchased from Sigma. Dichloromethane and ethanol with analysis grade were purchased from Shanghai Lingfeng Chemical

Reagent Co. Ltd, Shanghai .Carbon dioxide of 99.99% purity was purchased from Rui Li, Ltd. Shanghai.

2.2. Preparation of zein nano-particles

The SEDS experiment apparatus is shown in Fig 1. CO_2 from the cylinder (A) was condensed by a refrigerator and pumped into the precipitating vessel (G) by the piston pump (C) via the heat exchanger (D). CO_2 then went into the dual decompression vessel（H）from the precipitating vessel and gas flow rate was shown in gas flow meter（L）. After the pressure and temperature reached the desired values, the liquid solution was injected though inner part of the coaxial nozzle (I, Φinner = 100 μm, Φouter = 1000 μm) and spray with supercritical CO_2 which flow in the external pipe of the coaxial nozzle simultaneously. The flow of the liquid solution could be adjusted by an HPLC pump (E) and flow of the CO_2 could be adjusted by the piston pump (C). After all of the solution was injected in the vessel, $SCCO_2$ continued to be pumped into the vessel (G) to remove the remaining solvent, thus ensuring products contained rarely solvent. The organic solvent could be separated from CO_2 in the decompression vessel (H).

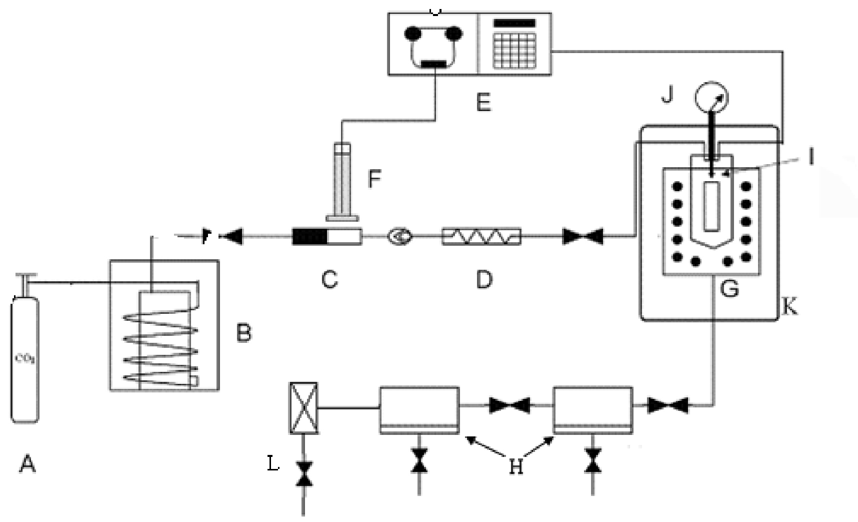


Fig 1

A-CO2 cylinder; B-cooling system(refrigerator);C-piston pump; D-heat exchanger; E-HPLC pump; F-solution; G-high pressure vessel(precipitator);H-dual decompression vessel; I-coaxial nozzle; J-pressure meter; K-incubator; L-gas flow meter

2.3. Adjusted coaxial nozzle

Nozzle is the innovation of this paper. The adjusted coaxial nozzle was shown in Fig.2. The relative position of the inner pipe (A) and the outer pipe (B) could be adjusted by regulating device (C). The coaxial nozzle can easily switch in two types (convex & horizon) due to the exist of the regulating device.

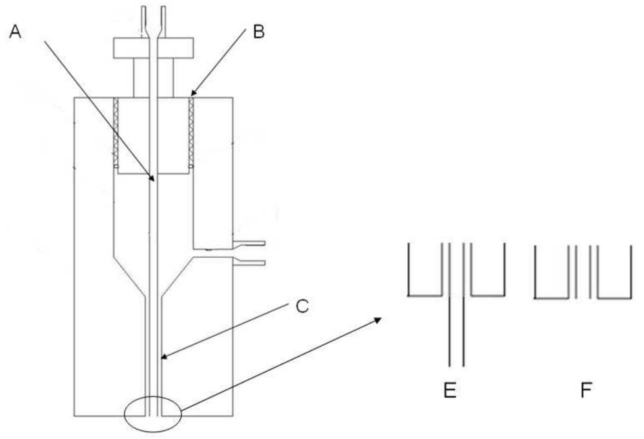


Fig 2

A- Inner pipe; B- Outer pipe; C- Regulating device; E- Convex position; F-Horizon position

2.4. Characterization

Morphological characterization of products which taken from vessel directly was observed by SEM (JOEL, JEM-7401F). A small amount of specimen was placed on one surface of a double-faced adhesive tape that sticks to the sample support and coated with gold under vacuum condition for about 20 s to enhance the electrical conductivity of samples. A JEOL JEM2010F(JEOL Ltd.) Transmission Electron Microscope (TEM) was also used to examine the structure of the nano-sized particles. The sample was dispersed in anhydrous ethanol and then loaded in the copper web.

2.5. CFD simulation

CFD calculations were performed using umoni2.0 COMSOL Mltiphysics 3.5 software. For simplification purposes we assume all fluids are incompressible, the simulations describe the mixing of pure SC-CO2 as the SCF phase and solvent as the liquid. The geometric model was based on the apparatuses which is an axial symmetry three-dimensional space. We established a two-dimensional graphics of the axis section as shown in fig3. The calculate region contains nozzle end(A) and the whole high pressure vessel(B). The details of the coaxial nozzle is expressed as inner pipe(C) and outer pipe(D).The generation of grid used an unstructured mesh method. It could imagine that the greatest change of flows is near the area where fluid flow out from nozzle, so this area dense grids take place.

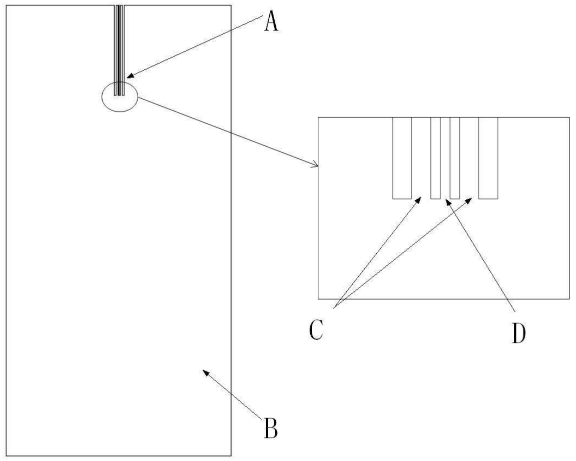


Fig 3 Geometric model

A-Nozzle; B-high pressure vessel; C-Inner pipe; D- Outer pipe;

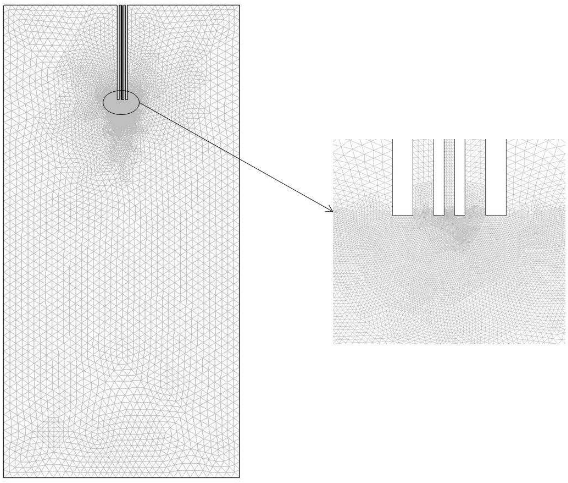


Fig 4 Unstructured mesh

Results and discussion

3.1 Formation of zein nanoparticles by SEDS

In order to reach the purpose of the research on the impact of the nozzle structures on the formation of zein nanoparticles, some operational parameters were fixed in the experiment shown in table 1 according to the preliminary experiments. The temperature and pressure of the processes were set beyond the mixture critical point (MCP) of the SC—CO_2 and the solvent. The mixed solvent of ethanol and dichloromethane was chosen with the volume ratio of 5:7.The concentration of the solution was 10mg/ml. The solution flow rate was 1ml/min.

Commentaire [y1]: 比例对吗？

Table 1 Some parameters fixed in the experiment

Temperature (□)	Pressure (MPa)	Concentration (mg/ml)	Solution flow rate (ml/min)	Solvent Ethanol:Dichloromethane
45	10	10	1	5:7

Table 2 showed the results of the experiments. As shown in the table, we obtained three different types of particle in morphology the size of the particle was from 50 to 350nm. Obviously when the nozzle had the same position, when flow rate of CO_2 increased the particle size got smaller, meanwhile the change direction of morphology is globule to rod like to fiber. When the CO_2 flow rate was fixed, nozzle position near horizon have the same trend in the effect of particles.

Exp. No.	CO_2 flow rate (kg/h)	Nozzle position	Morphology	Particle size (nm)
1	3	**Horizon**	Rod like	100-200
2	3	**Convex**	Globule	100-350
3	4.5	**Horizon**	Fiber	50-150
4	4.5	**Convex**	Rod like	100-200
5	6	**Horizon**	Fiber	Diameter<50
6	6	**Convex**	Fiber	Diameter≈100

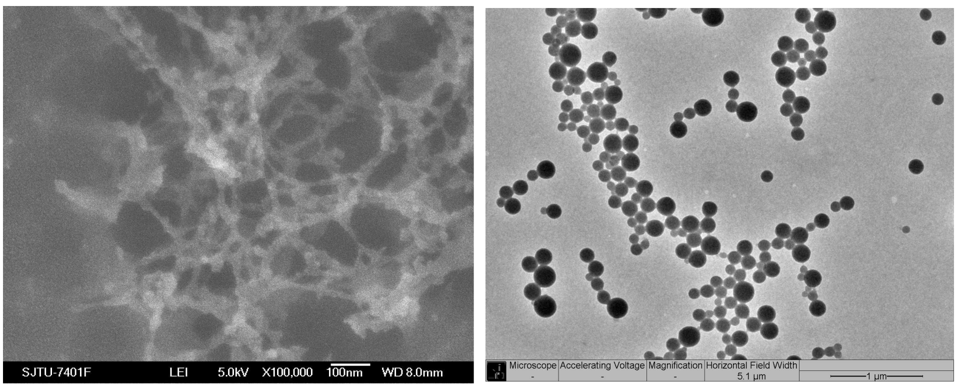
Table 2 Results of the experiments

3.2. The impact of nozzle structure –

3.2.1. Analysis of velocity field under different nozzle positions

As is shown in table 2, different zein particles in both size and morphology were obtained under different nozzle positions. It seemed that filamentous structure which showed in Fig 5 (a)(b) was appeared easily when the nozzle structure was the horizon style while rod like structure which showed in Fig 5 (c)(d) could appear in both nozzle positions. But the single spherical particles is only obtained under the condition that nozzle position is convex and CO_2 flow rate at 3kg/h through TEM image which showed in Fig.5(f). Because of different sample preparation methods, the SEM image which showed in Fig.6(e) shows these spherical particles aggregate together. Yet it also exhibits a wide size distribution of the particles. The apparent diameters are about 100 to 350nm, which is consistent with the results of Zhong et al. [22].

a b



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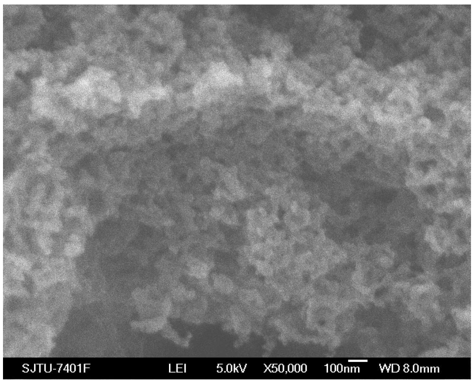

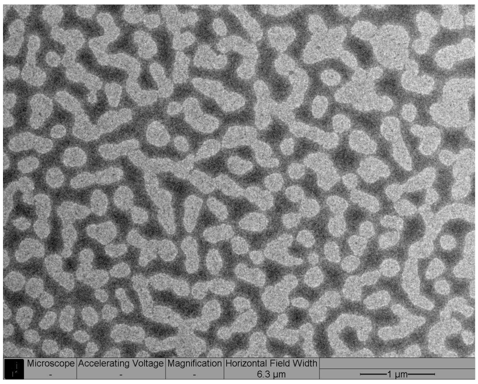

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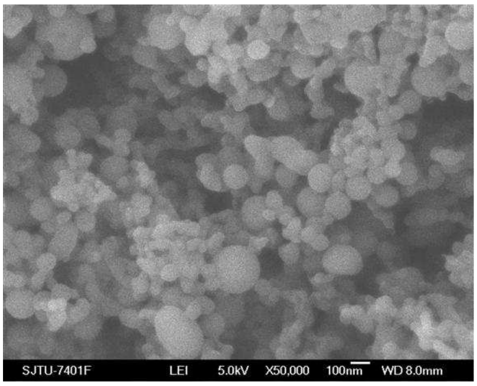

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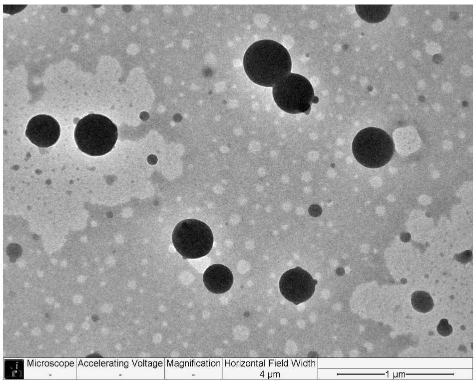
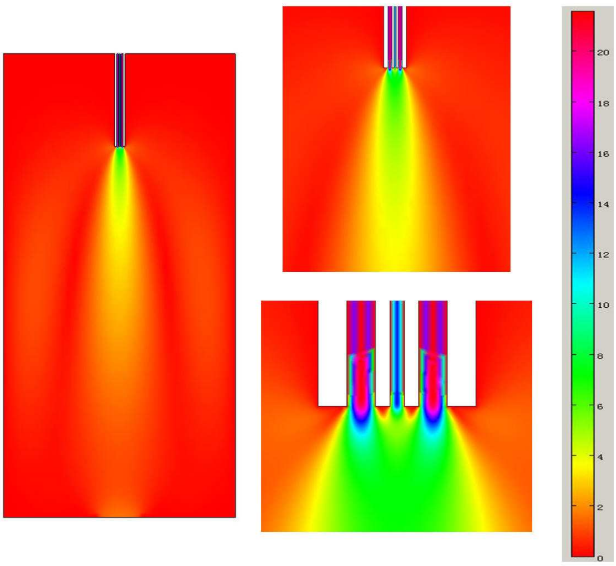


Fig 5 SEM & TEM image of zein nanoparticles obtained at 10 Mpa, 45 □ :
(a)(b)Horizon, CO_2 flow rate at 6kg/h;(c)(d) Convex, CO_2 flow rate at 4.5kg/h;

To explain the ~~above phenomenon~~results , a CFD model was established. The simulations of the experiments 1–6 of Table 2 were performed for the steady state analyzed by the software. The determined velocity fields of the experiment 3 and 4 ~~of Table 2 is~~are represented in Fig. 6 and ~~the velocity field for the experiment 4 is represented in~~ Fig. 7, respectively. The mean velocity changed rapidly near the jet as shown in Fig. 6 and ~~the velocity field for the experiment 4 is represented in~~ Fig. 7. The maximum velocity appeared in a very short distance from the jet. Just below the jet there was a region where the velocity decreased fast. It is believed that in this zone the turbulent mixing took place. Meanwhile the droplets were formatted in this region. It meant that the position of inner pipe affected the flow most, and the flow affected the formation of droplets thereby affected the formation of particles. However, the mean velocity of the most region in vessel was low, especially the spaces above the jet and far from it, which meant that these regions had no effect on the formation of particles.



Commentaire [h2]:

Fig 6. Velocity field

Nozzle position: Horizon; CO_2 flow rate: 4.5kg/h

~~Compared with Fig 4 and Fig 5,~~ Overall velocity field of No.3 and No.4 in Table 2 seemed similar looking from Fig.4 and Fig.5. ~~But~~However, we can find their differences if ~~when~~ we pay~~paid~~ attention to the region near jet, ~~differences appeared.~~ The velocity near the inner pipe in Fig 7 was lower than that in Fig 6.The ~~value~~ velocity nearly reduced double seen clearly from~~when we viewed~~ the ruler. It is noticed that the two models ~~had~~ have the same initial condition. Small changes in the nozzle structure caused significant changes in the velocity field especially near the jet,which~~. These changes~~ led to notable changes both on the size and morphology of the zein particles.

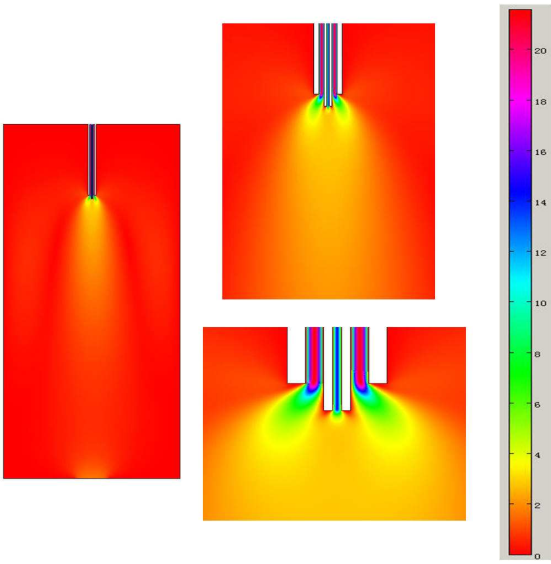


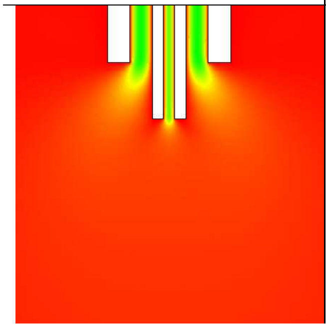
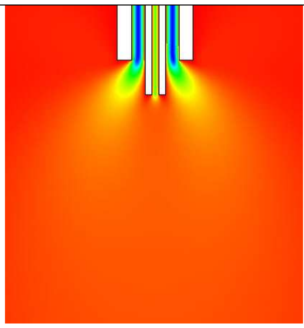
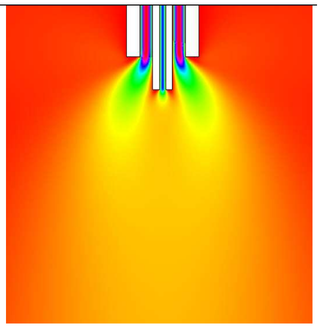
Fig 7. Velocity field

Nozzle position: Convex; CO_2 flow rate: 4.5kg/h

3.~~4~~2.2The effect~~impact~~ of flow rate of $SCCO_2$ under the same nozzle positions

Commentaire [y3]: 这节最好写为3.2.2节。此外，为什么仍然用图4和图5？从你现在的讨论中，几乎没有提到在相同的喷嘴结构下，流量是如何影响的？逻辑关系应该是：简要说明同一喷嘴下，流量不同的实验结果，然后用流场进行分析，说明异同。还可以用另一喷嘴的流场分析结果来进一步支持你的结论。

Table 3 Velocity field of convex condition at different CO_2 flow rate

a:3kg/h	b:4.5kg/h	c:6kg/h
		

As was shown in table3, ~~The~~ the maximum velocity value accrued in a very short distance from the jet. Just below the jet there was a region where the velocity decreased fast. When the flow rate of CO2 increased, the velocity gradient increased meanwhile the shear force grew. The droplets vanished in the region where the velocity decreased fast. In this area the main way of mass transfer is diffusion. ~~however~~ However in the area where the turbulent mixing took place the convection was dominated. So through the analysis of the convex nozzle ~~position~~ type, velocity field compare with experiment results, The size of particle decreased by the increasing of CO2 flow rate.

Commentaire [y4]: 这节是讨论位置不是流量，这句话何用？

Commentaire [y5]: 不清楚？ 应该是结合图 4 和图 5 来分析，找出他们流场的异同，从而指出颗粒不一样的原因，另外，在比较前，也应该先简要说明实验 3 和实验 4 的结果。然后进入 CFD 的讨论。

The generation of shear was due to the velocity difference between the two flows. The formation of droplets depended on the shear force based on spray theory. The stronger the shear was ,the smaller the droplets was. So a smaller droplets would formed when the flow rate of $SCCO_2$

increased. The decreased of particles size could be explained by the reduction of droplets.

The droplets were in regular shape when two-phase flow rate difference was small, thus with the increasing of the difference, shear force became stronger, the shape of droplets were irregular. The change in morphology of particles was considered as the alteration of the droplets.

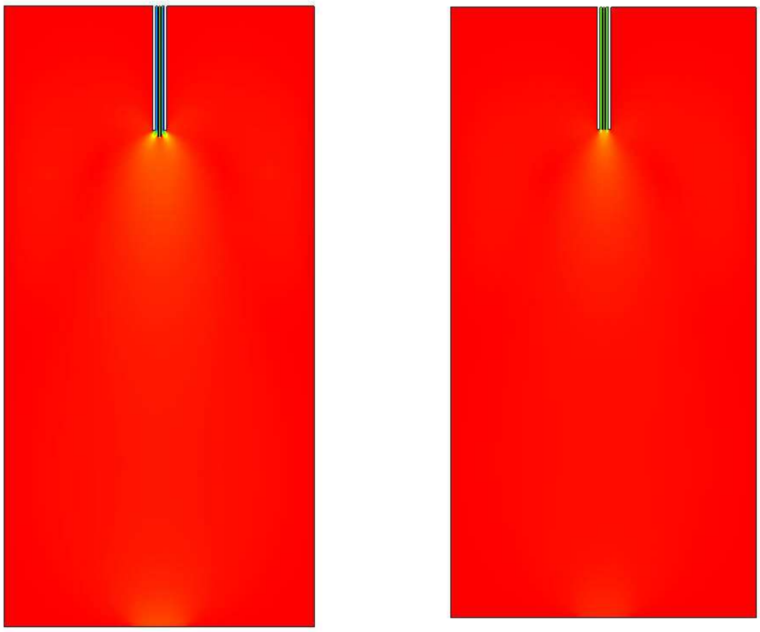


Fig 8. Velocity field

The left is in convex position while CO_2 flow rate at 4.5kg/h

The right is in horizon position while CO_2 flow rate at 3kg/h

Through the result of simulation(FIg 8.), a same flow field could be created by the combination of different flow rates and nozzle positions. Then, compared with the experiment results No.1 and No.4 in Table 2 we obtianed similar particle under different nozzle structure and flow rate of

Commentaire [y6]: 那个实验？

supercritical fluid for the similar flow field. In addition we proved indirectly that the small region under jet played a decisive role in the formation of particles at the same thermodynamic conditions.

Commentaire [y7]: 这段分析讨论的逻辑关系应该是：首先简要说明表 2 中的相关实验结果，然后比较两种情况下的流场关系，说明异同的原因，最后还可以进一步说明当流量变化时的实验结果，目的是进一步支持流场分析的结果。请把 3.2.1 重新组织整理。

Conclusion

The results demonstrate that the nozzle structure in one of the factors of particle formation in SEDS process. A small alteration in nozzle structure would led a big change in the shape and size of the particles.

CFD calculations prove that the shape of flow field are determined by both nozzle structure and the velocity of two phase(especially supercritical fluid). Compared with experiment results, the particles form similar morphology and size when the shape of flow field near solution jet is similar.

Three types of zein nano structure were obtained by our device. The fiber structure is first found and it may has potential usage in many fields.

Acknowledgements

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