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Application of Spontaneous Suction Phase-Dispersing (SSPD) Extractors in the Extraction of Penicillin G

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Abstract

The extraction of penicillin G from an aqueous solution with butyl acetate (BA) and tributyl phosphate (TBP) as extractants was carried out at pH 4 with spontaneous suction phase-dispersing (SSPD) extractors under various operating conditions. Four kinds of SSPD extractors were tested with results compared to those obtained by using an extractor with mechanical stirrers. Rotation speed and different extraction systems were found to influence the penicillin recovery and the stability of emulsion formed during extraction. The percentage of extraction under optimum conditions was 91% without formation of emulsion. The laser particle size measurement instrument combined with SSPD can be used to measure the emulsion droplet size *in situ*.

Index Entries: Spontaneous suction phase-dispersing (SSPD); reactive extraction; penicillin G; emulsion droplet size, *in situ*.

Introduction

Penicillin G is a weak acid. The pH of extraction is generally carried out at 2–2.5 in order to extract the penicillin G in its undissociated molecular form. The penicillin G containing solution was cooled down to 10°C before extraction to reduce the decomposition from its instability at low pH *(1)*. The traditional extraction has been performed in a mixer–settler *(2)* or countercurrent extraction decanter *(3,4)*. To avoid the losses, reactive extractions with secondary amines *(5)* and neutral phosphate esters *(6)* have been

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studied. The extraction can then be carried out with extraction at pH 3–4 and stripping at pH 6.5–7.2 *(6)*. On the other hand, compared with BA extraction, only a quarter amount of demulsifer is needed during processing by using tributyl phosphate (TBP) in kerosene as extractant.

The formation of rather stable emulsion from the extraction of penicillin G from fermentation broth often causes problems during extraction, leading to a significant increase in production cost. Centrifugal extractors are normally used instead of simple mixer–settlers, partly from the advantage of breaking emulsion. In spite of this, emulsion is still the limit for increasing the extractor throughput and causes the loss of products and solvent *(7)*. Many kinds of effective demulsifiers have been developed to eliminate or decrease the emulsion *(8,9)*, but little work has been published on the development of novel and effective extractors for penicillin extraction from fermentation broth.

Spontaneous suction phase-dispersing (SSPD) equipment has been used in the gas–liquid systems for fermentation processing, and its lower shear force and dispersing capability make it more suitable for the case of handling biological systems that are easy to emulsify. SSPD extractors can suck the gas or liquid through the holes on the side or at the end of the hollow rotating shaft. SSPD extractors can also avoid the violent agitation and strong shear force; moreover, they can disperse the light (or heavy) phase into the heavy (or light) phase continuously to carry on the extraction process. In many cases studied, emulsification is practically avoided. The phase-dispersing extractors have been used for the extraction of penicillin G from the filtrate solution obtained from fermentation and also some other easy to emulsify systems during extraction process *(10,11)*.

Likewise, because SSPD extractor can avoid the strong agitation, laser particle size measurement instrument combined with SSPD extractor can measure the emulsion droplet size distribution *in situ* during the penicillin extraction *(12)*. After adding some protein as emulsifier or adding some commercial demulsifier, we can measure the droplet size of the emulsion formed *in situ* and study the mechanism of emulsion and demulsion.

Materials and Methods

Reagents Used

Simulated aqueous solutions were prepared by mixing 0.05 mol/L penicillin potassium salt and 1 mg/mL whey protein or albumin. Fresh fermentation filtrate was kindly supplied by North China Pharmaceutics Company, which were a mixture of penicillin G, organic acids, coloring, and other impurities. Compared with the simulated penicillin solution, the fermentation filtrate, a real sample of filtered fermentation broth, was prepared by the upstream fermentation production unit. Organic phase was BA or BA containing 5.7% TBP (wt %). D925m T_2 was the type of demulsifier used, which was a mixture of four components: amphiphilic surfactant as the main component, non-ionic surfactant as a minor component, wetting

agent, and dispersant. Low dosage was required and high-water solubility was its prominent property. It was made by Zhongke Company of our research institute.

Experimental Apparatus

The concentration of penicillin was measured by WZZ-2A automatic polarimeter manufactured by Shanghai Physical Optics Apparatus Plant. Mono-layer flat quatrefoil stirrer or tri-level flat quatrefoil stirrer used with single-channel SSPD extractor or three-channel SSPD extractor was designed in our laboratory. An improved sample cell of the Malvern 2200 laser particle instrument was also designed and made in our laboratory *(10,12)*.

SSPD Extractors and Instrument for Measuring the Emulsion Droplet Size

The principal parts of four kinds of SSPD extractors used are labeled in Fig. 1. The conformation of single-channel upper-phase SSPD as shown in Fig. 1A consists of three parts: the hollow Teflon cylinder with 30 small bores on the periphery connected with the hollow stainless-steel pipe with a big hole on its side, and a Fluon pedestal connected with the hollow pipe. When the hollow pipe is rotated, the top layer liquid will be sucked into the hollow pipe and dispersed into the bottom continuous phase as droplets through the small bores. The extraction process with formation of emulsion would occur when the two liquid phases came into contact. The principle of single-channel lower-phase SSPD extractor as shown in Fig. 1B is the same as single-channel upper-phase SSPD extractor. The difference is that no hole is drilled on the side, and the liquid phase is sucked into the shaft at the end of the hollow stainless-steel pipe. When it is rotated, the lower aqueous phase will be sucked into the hollow shaft through the end of the pipe and dispersed into the upper continuous organic phase. Figure 1C and 1D indicate the three-channel upper- and lower-phase SSPD extractors. Compared with Fig. 1A, the three-channel upper-phase SSPD extractor has two cylindric sleeves connected with the hollow pipe as shown in Fig. 1C. Thus, two additional channels are formed for liquid flow. For each cylindrical sleeve, 30 small bores were drilled on its lower part. When it is rotated, the upper organic phase would be sucked into the lower aqueous phase through the three channels just mentioned. The three channels provide higher throughput, which will result in faster exchange of fluid than only a single channel. As shown in Fig. 1D, three-channel lower-phase SSPD extractor has the same set up as in Fig. 1C, except the final set up installed is similar to Fig. 1B instead. When it is rotated, the lower aqueous phase would be sucked into the three channels and dispersed into the upper continuous organic phase. The actual size of the SSPD extractors used in this work was 3 cm in diameter and 15 cm high. The glass vessel was 10 cm high and with 5.5 cm diameter. During extraction, the liquid inlet and outlet must be in different liquid phases. For example, for the upper-phase SSPD extractor shown in Figs. 1A and 1C, the inlet of the channels should be in the organic phase with the outlet in the aqueous phase.

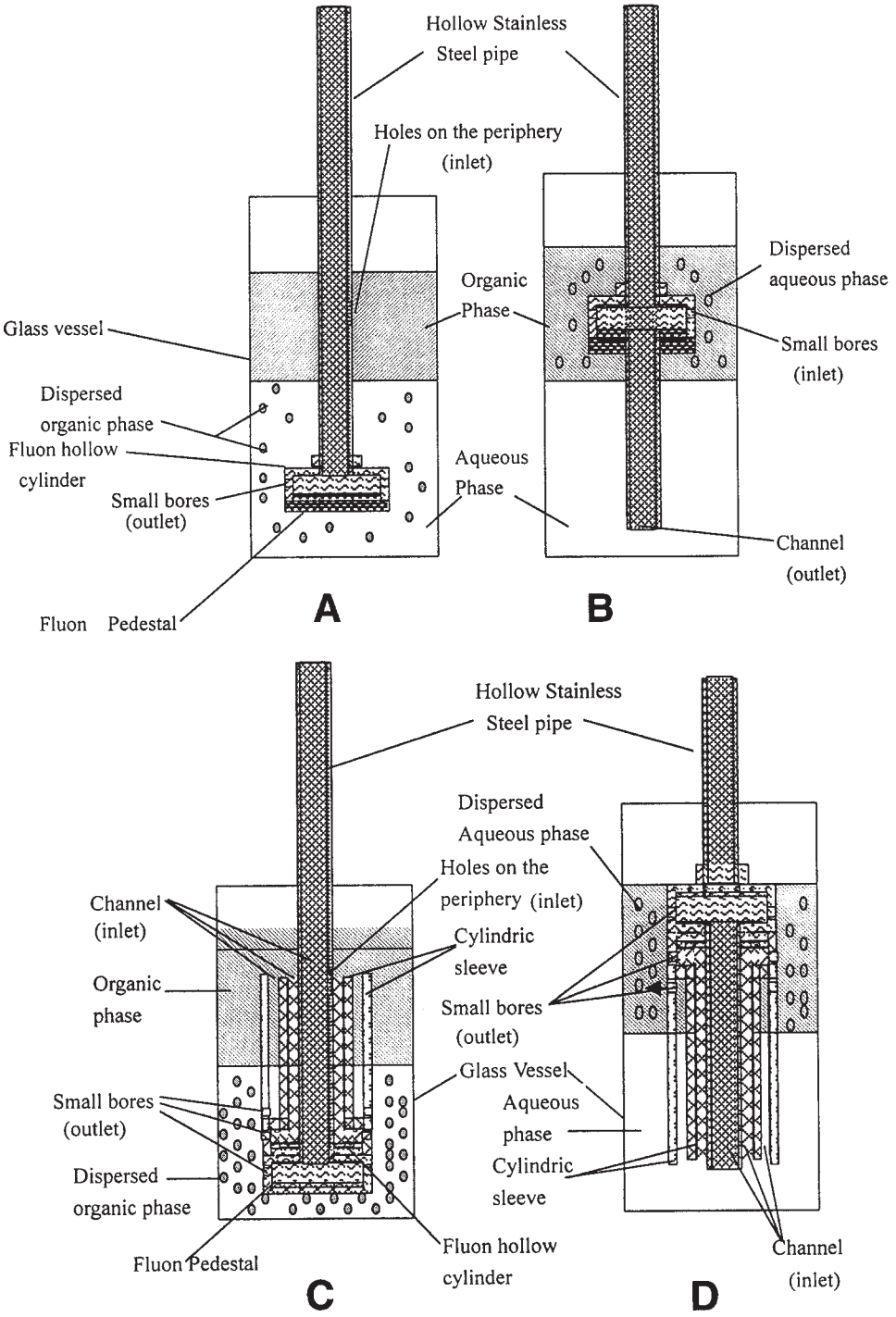

Fig. 1. Schematic representation of spontaneous suction phase-dispersing (SSPD) extractors: **(A)** single-channel upper-phase SSPD extractor; **(B)** single-channel lower-phase SSPD extractor; **(C)** three-channel upper-phase SSPD extractor; and **(D)** three-channel low-phase SSPD extractor.

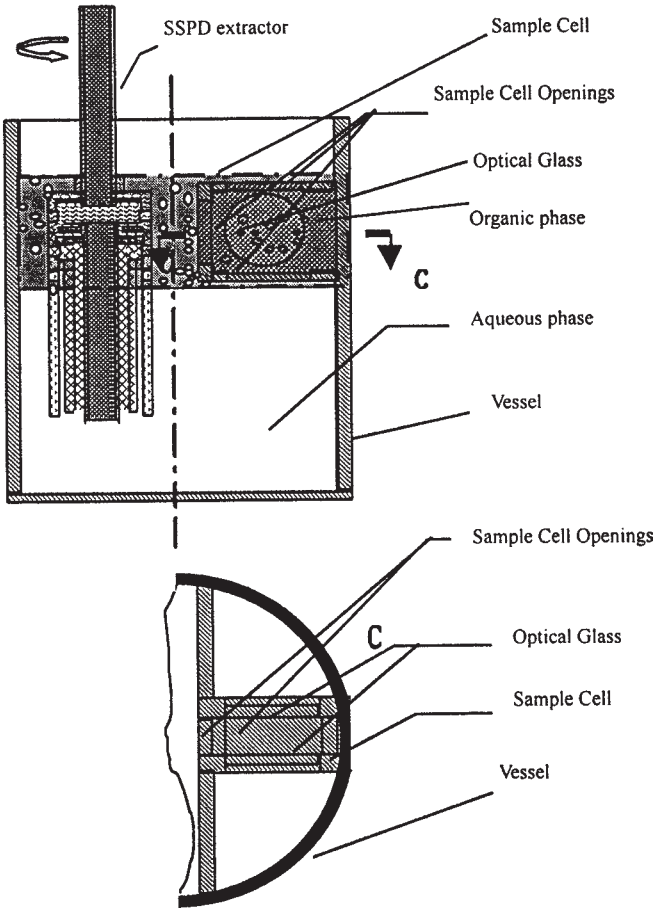

Fig. 2. Schematic drawing of the vessel used for measuring the droplet size in organic phase.

The schematic drawing of the vessel used for measuring the droplet size of emulsion *in situ* is shown in Fig. 2. The sample cell was inserted into the stainless-steel vessel. Three kinds of openings were set up at the top, bottom, and left-hand side. From the openings of the sample cell, the inner part of the sample cell was linked with the outside. When the SSPD extractor is rotated, the lower aqueous phase would be dispersed into the sample cell, and the droplet size formed in the organic phase will be recorded by the instrument *in situ*. The stainless-steel vessel was 15 cm high and 15 cm in diameter. The total set up for organic phase emulsion droplet size measurement during the experiment is shown in Fig. 3. The average droplet size as surface mean diameter (SMD) was given by the computer program of the instrument. The average droplet size SMD was calculated by the formula: SMD = $(\sum f_i d_i^2)^{0.5}$, $f_i = n_i/\sum n_i$, f_i is the number percentage of the droplet whose diameter was d_i in droplets measured. The relationship between the average droplet size SMD with time was shown in Fig. 9.

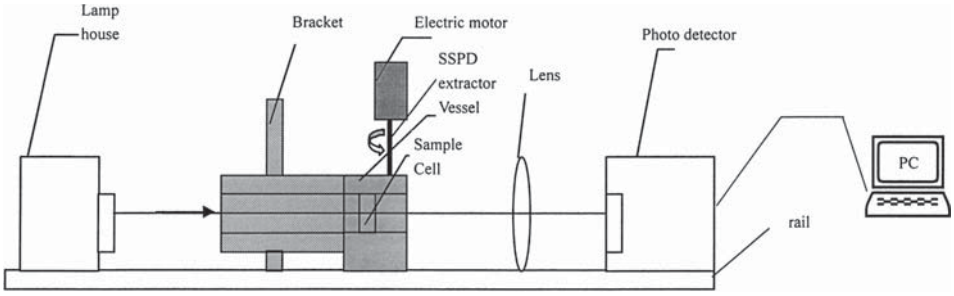

Fig. 3. The schematic drawing of modified Malvern 2200 laser particle size measurement instrument.

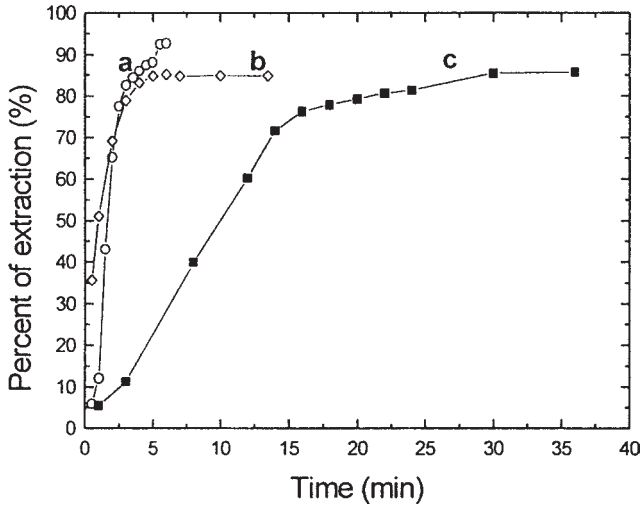

Fig. 4. Percentage of extraction of penicillin at 300 rpm. **(A)** From fermentation filtrate by three-channel upper-phase SSPD extractor. **(B)** From simulated aqueous solution by three-channel upper-phase SSPD extractor. **(C)** From simulated aqueous solution by tri-level flat quatrefoil stirrer.

Extraction and Droplet Size Measurement

Forty milliliters of BA containing 5.7% TBP (wt %) and 80 mL simulated aqueous solutions or fresh fermentation filtrate were put into the glass vessel. The SSPD extractor and flat quatrefoil stirrers were used to extract penicillin under different rotational speeds. The upper organic phase was then taken out for optical measurement at a certain time interval as required. When emulsification occurred with using flat quatrefoil stirrers, centrifugation and D925mT_2 demulsifier were used to separate the two clear phases before optical measurement. The relationship between percentage of extraction with time is shown in Figs. 4–6 for different experimental conditions. The extraction equilibrium time and emulsification sta-

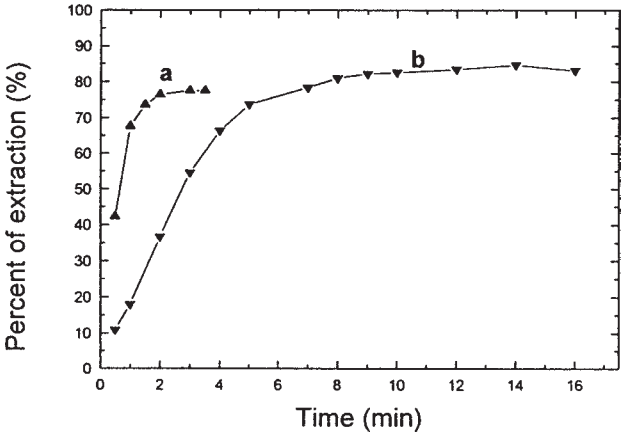

Fig. 5. Percentage of extraction of penicillin from simulated aqueous phase at 600 rpm. **(A)** By three-channel upper-phase SSPD extractor; **(B)** by single-channel upper-phase SSPD extractor.

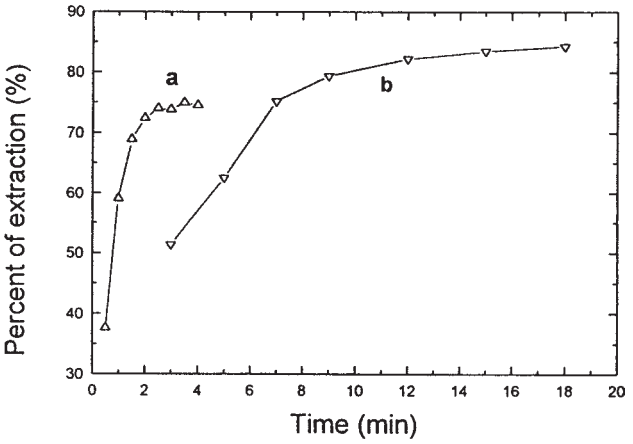

Fig. 6. Extraction of penicillin from simulated aqueous solution at 600 rpm. **(A)** By three-channel lower-phase SSPD extractor; **(B)** by single-channel lower-phase SSPD extractor.

tus are listed in Table 1. All the experiments were carried out at room temperature.

For measuring the emulsion droplet size distribution *in situ*, 400 mL BA and 800 mL simulated aqueous solution were carefully added into the stainless-steel vessel. The aqueous phase was first put into the vessel, then the organic phase was carefully added along the vessel side. The three-channel upper phase SSPD agitation set up was put into the vessel. The sample cell was only immersed in the organic phase. After 3 min of rotation of the SSPD agitator, the percentage of distribution of droplet size formed in BA was shown in Figs. 7 and 8 for six different systems tested.

Table 1
Comparison of Time Required for Extraction Equilibrium and Emulsification Status by Using Different Mixing Set-Up

Extractors	Rotation speed (rpm)	Extraction equilibrium time (min)	Emulsification and phase splitting status
Mono-level flat quatrefoil stirrer	300	>120	No emulsion formed
Tri-level flat quatrefoil stirrer	300	30	Emulsion centrifugal force and 50×10^{-6} mol/L T_2 demulsifier were needed to demulsify.
Single-channel upper-phase SSPD extractor	600	9	The two phases were separated by standing, but interfacial layer is somewhat blurry.
Three-channel upper-phase SSPD extractor	300	8	Two phases were separated completely in 10 s by standing.
Three-channel upper-phase SSPD extractor	300	8[a]	Two phases were separated completely in 10 s by standing.
Three-channel upper-phase SSPD extractor	600	2	Two phases were separated completely in 25 s by standing.
Three-channel upper-phase SSPD extractor	600	2[a]	Slightly emulsify, the two phases were separated by centrifugation
Single-channel lower-phase SSPD extractor	600	12	Emulsion, the two phases were separated by centrifugation
Three-channel lower-phase SSPD extractor	600	3.5	Two phases were separated completely in 21 s by standing.

[a]Represents fresh penicillin fermentation filtrate system.

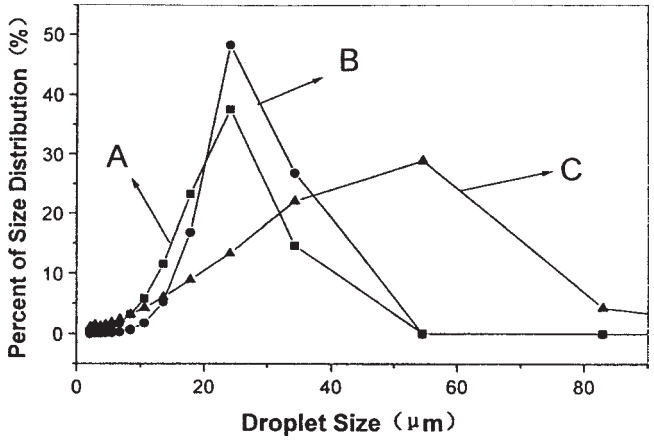

Fig. 7. Droplet size distribution of three different albumin systems after 3 min stirring. **(A)** Albumin in water; **(B)** albumin in 10 m*M* KCl; **(C)** albumin with D925m T_2.

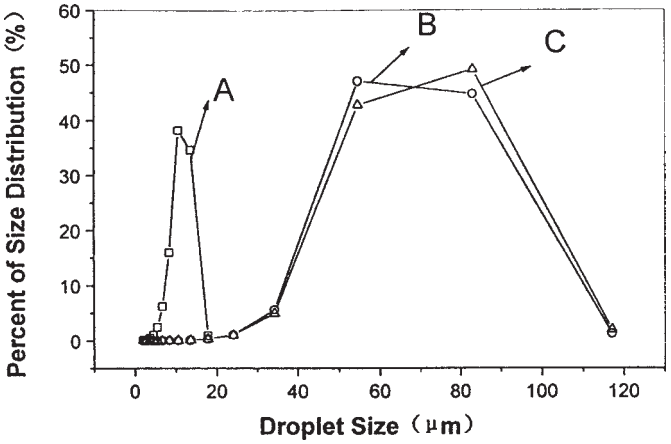

Fig. 8. Droplet size distribution of whey protein and D925m T_2 systems after 3 min stirring. **(A)** Whey protein in water, **(B)** 50×10^{-6} mol/L D925m T_2 in water; **(C)** whey protein in 50×10^{-6} mol/L D925m T_2 solution.

For measuring the change of droplet size with penicillin extraction, 400 mL BA containing 5.7% TBP (wt %) and 800 mL simulated aqueous solutions were added into the vessel. The solution pH was adjusted to 4.0 by adding 10% H_2SO_4. During the extraction process, the percentage of distribution of droplet size in the organic phase was recorded every minute.

Results and Discussion

Some important criteria were used to evaluate SSPD extractor, such as percentage of extraction, equilibrium time, and phase separation phenomenon. From Fig. 4A and Table 1, it was found that the equilibrium percentage of extraction of penicillin fermentation filtrate was 91% with 8 min to

reach the extraction equilibrium, while 8 s standing was required for separating the two phases into transparent liquid phases at 300 rpm in the three-channel upper-phase SSPD extractor. Curves b and c of Fig. 4 and Table 1 showed that 30 min was required to reach the extraction equilibrium for tri-level flat quatrefoil stirrer and only 2 min was required for three-channel upper-phase SSPD extractor with a percentage of extraction of 86%. Moreover, with three-channel upper-phase SSPD extractor, no emulsification was observed.

It was shown in Fig. 5 and Table 1 that both single-channel and three-channel upper-phase SSPD extractors did not induce emulsification with simulated aqueous solution at 600 rpm, only slight blurring occurred for single-channel upper-phase SSPD extractor. Because the fluid flux in three-channel extractor was much higher than that in single-channel extractor, the time to reach extraction equilibrium with the three-channel extractor was only about one-fourth of that for single channel. The percentage of extraction was about the same.

It is shown in Fig. 6 and Table 1 that single-channel lower-phase SSPD extractor caused emulsification, but three-channel lower-phase SSPD extractor did not induce emulsification at 600 rpm. Three-channel lower-phase SSPD extractor reduced the extraction equilibrium time to one third of that with a single-channel one, but the percentage of extraction was lower than that obtained with a single-channel agitator. From Figs. 5 and 6 show the faster rate of extraction and higher percentage of extraction with upper-phase SSPD extractor than those obtained with lower-phase SSPD extractor.

From Fig. 7A, the emulsion formation with albumin solution was started after 3 min stirring. When the D925m T_2 was added, the result (Fig. 7C) showed that the droplet size became larger and the BA phase became clearer. As the droplet size is larger for Fig. 7C than that shown in Fig. 7A, the droplet would be unstable, and demulsification occurred. Figure 7B showed that the presence of KCl had no measurable effect on the droplet size.

Comparing Fig. 8A with Fig. 7A we found that the droplet size of the most probable percentage of distribution for whey protein solution was 12 μm less than that of the albumin (21 μm), which agreed with experimental results that the whey protein in organic and aqueous phase was easy to emulsify in comparison with albumin *(10)*. Comparing curve A with B of Fig. 8, it was found that the droplet size distribution of whey protein in water solution was almost the same as D925m T_2 in water solution. It was deduced that the interfacial property of D925m T_2 took the dominant role in water/oil emulsion system. D925m T_2 can increase the droplet size. This was the part of reason of demulsification by using the demulsifer in penicillin G extraction. From Fig. 9, it was found that with increase of penicillin extracted into the organic phase, the droplet SMD size in organic phase increased. The results indicated that the presence of penicillin acid in organic phase resulted in the increased droplet size and decreased emul-

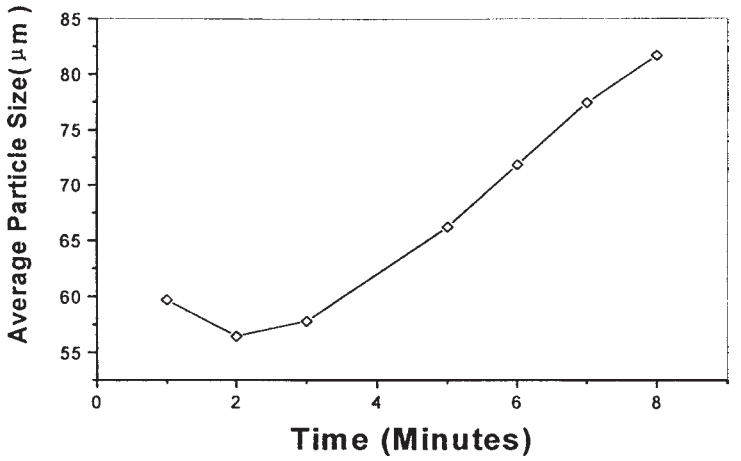

Fig. 9. The change of average droplet size SMD during penicillin extraction in 1 mg/mL whey protein.

sion formation. This statement was consistent with the interfacial tension experimental results *(10)*. With more penicillin extracted into the organic phase, the interfacial tension between the organic phase and the aqueous phase decreased continuously.

Conclusions

At pH 4, denature of proteins induced by acid was weakened greatly. At the same time, SSPD extractor can effectively extract penicillin G, plus the effect of avoiding the emulsification caused by strong shear force and violent agitation. The extraction of penicillin G in SSPD extractors is probably an effective method for demulsification. The SSPD extractor with rotation at a reasonable speed could still maintain the aqueous/organic interface. The laser particle size measurement instrument combined with SSPD extractor can measure the emulsion droplet size distribution *in situ*, and it will be possible to provide a method to study the emulsion and mechanism of demulsion.

Acknowledgments

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