Regular Article

Reconstitution of L-Asparaginase in Siliconized Syringes with Shaking and Headspace Air Induces Protein Aggregation

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The aim of this study was to characterize protein aggregation during reconstitution of a highly concentrated solution of lyophilized L-asparaginase (L-ASP). The effect of the preparation method on L-ASP aggregation using siliconized or non-siliconized syringes and the effect of storage after preparation were evaluated by far-UV circular dichroism spectroscopy, Raman microscopy, flow cytometry, and flow particle image analysis. To investigate the effect of syringe type in combination with shaking and headspace air on L-ASP aggregation, four kinds of L-ASP in 5% glucose solutions were prepared (in the presence or absence of silicone oil and headspace air). Slight differences in L-ASP secondary structure were observed between the siliconized and non-siliconized syringe systems before shaking. Large numbers of sub-visible (0.1–100 µm) and submicron (0.1–1 µm) particles were formed by preparation with siliconized syringes and the combination of shaking and headspace air. The number of aggregated particles was not decreased with increased storage time. The Raman microscopy, flow cytometry and flow particle image results suggested that L-ASP interacted with silicone oil, which induced aggregation. Nevertheless, sub-visible and submicron particles were also formed with non-siliconized syringes. However, using non-siliconized syringes, the number of aggregated particles decreased with storage. No changes in particle character were observed before or after shaking with headspace air in non-siliconized syringes, indicating that soluble aggregates formed and dissolved with storage. Silicone oil in syringes, in combination with shaking and headspace air, strongly affected the aggregation of lyophilized L-ASP formulations during preparation.

Key words protein aggregation; silicone oil; L-asparaginase (L-ASP); sub-visible particle; flow cytometry; Raman microscopy.

Various therapeutic proteins have been commercialized owing to recent advances in biotechnology and formulation. These therapeutic proteins are used for the treatment of numerous diseases including cancer, autoimmune diseases, and diabetes mellitus. However, it has been reported that protein aggregates in these products have caused side effects, immunogenicity, and allergic reactions.1–3) Substantial effort has been expended to develop stable formulations to prevent these events. These stable formulations are necessary for safe and effective pharmaceutical therapies.

There has been great interest in the external stress affecting protein stability during production, filling, shipment, storage, and final administration.4–6) These external stressors include temperature changes, lyophilization, rehydration, oxidation, and light exposure.5–7) Interactions between proteins and foreign materials, such as rubber, metal particles, and administration devices, can also occur.8–12) For injectable formulations, syringes (glass or polypropylene) and needles are used for reconstitution and administration. In these devices, silicone oil is used as a lubricant for ease of handling. Recently, several authors reported that silicone oil induced protein aggregation.13–16) Jones et al. showed that aggregation of model proteins occurred with the addition of 0.5% (w/v) silicone oil during incubation at elevated temperatures.17) Additionally, Thirumangalathu et al. reported that silicone oil induced the aggregation of a monoclonal antibody in aqueous solution, and that polysorbate 20 was a useful additive for inhibiting aggregation.18)

The size of protein aggregates varies. Aggregate sizes can range from dimers in the nanometer range to larger aggregates of hundreds of microns, which are visible to human eye. Based on the current classification of protein aggregates and particles, particles with a size above 0.1 µm can be further classified into sub-visible (0.1–100 µm) and visible particles (above 100 µm). Particle sizes from 0.1 to 1 µm were categorized as submicron particles.19) It is recommended that different analytical methods be combined for each sample to overcome the limitations of using a single method to determine size range, concentration range, or delivered parameters.20,21)

In clinical situations, final protein products are frequently diluted or rehydrated with intravenous fluids to achieve the appropriate dose, and the prepared injection is administrated to the patient. In Japan, siliconized syringes and needles are used for the preparation of injectable medication, since non-
siliconized syringes and needles were not approved for use in the preparation of injectable medications by the Ministry of Health, Labour, and Welfare of Japan. However, in 2014, non-siliconized free syringes were approved by the Japanese government for medical use. Nevertheless, non-siliconized syringes are used less frequently for the preparation of injectable medication.

L-Asparaginase (L-ASP) is an enzyme used for the treatment of acute leukemia. L-ASP is a tetramer of four identical subunits (321 amino acids each) held together by non-covalent forces. Since L-ASP is derived from bacteria, it has the potential to be immunogenic. Hypersensitivity reactions to L-ASP occur in up 30% of patients, and are frequently associated with the development of neutralizing antibodies. Although L-ASP is typically administered intravenously, intramuscular and subcutaneous administration of L-ASP has been used to avoid hypersensitivity reactions.

In Japan, pharmacists frequently prepare injectable drugs, including anticancer drugs. Generally, high concentration protein solutions have the potential to aggregate, since the distance between protein molecules is decreased, resulting in increased intermolecular interactions between protein molecules.

The aim of this study was to investigate the aggregation of highly concentrated L-ASP solutions during preparation. The effects of shaking, air headspace, and silicone oil on aggregation were also investigated. In interview form, it is written that L-ASP injection should be prepared before injection for the formation of aggregate product. However, prepared L-ASP injection might be stored for several hours in actual clinical situation. Therefore, the effect of storage on the aggregated samples was evaluated. Protein particles have soluble and insoluble characteristics, so it is important to characterize visible and sub-visible proteins in situ. Therefore, a micro-chamber was developed, composed of a closed cell system designed to entrap the protein particles. Entrapped protein particles in the micro-chamber were analyzed by Raman microscopy to determine the particle characteristics.

A flow particle imaging analyzer (FPIA) was used to evaluate protein aggregation, in order to determine the count and size of protein particles in the sub-visible range. A flow cytometer was used to detect the particles in the sub-visible and submicron ranges. Far-UV circular dichroism (CD) spectroscopy was used to evaluate the secondary structure of L-ASP and the interaction between L-ASP and silicone oil in solution for particles in the soluble range.

### Experimental

**Materials** Commercially available L-ASP injections (LEUNASE® Injection, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) and 5% glucose (dextrose) solution of injectable grade (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) were used. Siliconized syringe with butyl rubber plunger were used for the Terumo product (TERUMO CORPORATION, Tokyo, Japan). NORM-JECT® syringes are used as non-siliconized syringe with containing no rubber tip on plunger (Henke-Sass, Wolf, Tuttingen, Germany). All other chemical reagents were of analytical grade.

**Sample Preparation** A lyophilized formulation of 10000 KU L-ASP was dissolved in 1 mL of 5% glucose solution in 5 mL siliconized or non-siliconized syringes. To investigate the effect of the air and silicone oil on the syringe, four samples were prepared by different preparation processes. Their preparation methods are summarized in Table 1.

Samples 1 and 2 used siliconized syringes during preparation, and 4 mL of air headspace was added to the syringe in Sample 2. Non-siliconized syringes were used for Samples 3 and 4. In Sample 4, 4 mL of air headspace was added to the non-siliconized syringes. Syringes containing L-ASP solution were shaken with mechanical shaker (SR-1, TAITEK, Saitama, Japan) for 5 min at a shaking speed of 250 rpm. To evaluate the dissolved L-ASP solution before shaking, dissolved L-ASP solutions with siliconized or non-siliconized syringes were collected by pipet from the vial after removal of the vial cap and used for analysis.

**Micro-chamber** Figure S1a shows a photograph of the micro-chamber (INT-700, INTOROTECH Co., Ltd., Kanagawa, Japan), a closed cell system used to entrap the protein particles.

The sample solution containing the protein aggregates was encased in a chamber composed of a 5 mm thick O-ring sandwiched between two pieces of quartz glass (diameter: 1.0 cm). In order to introduce a sample into the micro-chamber cell, the sample solution was infused into the cell from the inlet hole on the side of the cell with a syringe and needle (Fig. S1b). At the same time, another needle was inserted into the

### Table 1. Summary of Sample Preparation Method

| Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|----------|----------|----------|----------|
| Syringe type | Siliconized syringe | Siliconized syringe | Non-siliconized syringe | Non-siliconized syringe |
| Headspace air in the syringe during preparation | – | + | – | + |

### Table 2. The Percent Relative Abundance of Protein Species in L-ASP Solution Prepared with Siliconized and Non-siliconized Syringe from SEC Analysis

| Formulations | Total | Monomer | Oligomer |
|--------------|-------|---------|---------|
| | Peak area (%) | S.D. | Peak area (%) | S.D. | Peak area (%) | S.D. |
| L-ASP solution prepared with siliconized syringe | 91.9 | ±0.6 | 74.4 | ±0.5 | 17.5 | ±0.1 |
| L-ASP solution prepared with non-siliconized syringe (as control) | 100.0 | ±0.9** | 80.9 | ±0.7** | 19.1 | ±0.2** |

Data represents the mean±S.D. (n=3). ** Significant at *p*<0.01 compared with siliconized syringe system in same column.
outlet hole to remove the air from the micro-chamber. After sample preparation, Raman microscopy was used to evaluate protein aggregation.

**Flow Cytometry Analysis** A BD Floreescence Activated Cell Sorting (FACS) Cant II Flow Cytometer (Becton-Dickson, San Jose, CA, U.S.A.) with forward-scattering (FSC) and side-scattering (SSC) was utilized. The detectable particle range for this apparatus is 0.5 to 50 µm. All flow cytometry data sets were collected using BD FACS flow sheath fluid. For each detector, sensitivity and gain were optimized to maximize the detection of existing particles. Samples were analyzed with a middle flow rate (60 µL/min) for 10 s after filtration with a nylon filter (mesh size: 70 µm). Polystyrene bead suspensions with diameters of 0.5 and 3.0 µm were diluted with 150 mM sodium phosphate buffer at a ratio of 1:2000 and measured by flow cytometer to speculate the particle size. Data obtained were analyzed by Flow Jo version 8.7.3 (Tree Star, Ashland, OR, U.S.A.).

**Far-UV CD** Far-UV CD spectra were recorded from 180 to 250 nm using a Jasco J-600 CD spectrometer (Jasco International, Tokyo, Japan). Analyses were performed in a 1 mm path length quartz cuvette at 25°C using a scan rate of 5 nm/min, a response time of 8 s, and a bandwidth of 1 nm. L-ASP in 5% glucose solution was prepared at a 100-fold dilution with phosphate-buffered saline (pH 7.4) for the measurement. Each spectrum was corrected for background with the spectrum of the corresponding buffer. The CD signals were converted to molar ellipticity per amino acid residue.

**High-Performance Size Exclusion Chromatography (HP-SEC)** The samples were analyzed using the Shimazdu high-performance liquid chromatography (HPLC) system (Shimadzu, Tokyo, Japan), with simultaneous UV absorbance detection at 280 nm with a TSK Gel 3000 SWXL (300 nm×7.8 mm)
column (Tosoh Bioscience, Tokyo, Japan). The running buffer was composed of 100 mM phosphate, 100 mM Na₂SO₄, and 0.05% NaN₃ at pH 7.1. Ultraviolet (UV) detection was performed at 280 nm. Three microliter of aliquots were injected into the HPLC system. Flow rate was 0.5 mL/min and elution time was set at 30 min.

To calculate the protein recovery, the total area under the curve (AUC) of the UV absorbance signal at 280 nm of the stressed samples was compared with the total AUC of the L-ASP solution prepared with non-siliconized syringes, which was set to 100%.

**Flow Particle Image Analyzer (FPIA)** A Sysmex FPIA-3000 (Malvern Instruments Japan, Hyogo, Japan) was used to acquire the distribution data for both particle size and shape. Samples were passed through the measurement aperture where images were captured using stroboscope illumination and a charge coupled device camera. Digital images of the particle samples were collected, and multiple parameters of each particle were calculated by the FPIA-3000 software. To obtain precise values, the Sysmex FPIA-3000 was calibrated before conducting experiments with a certified size standard, an aqueous suspension of polymer microspheres with a diameter of 2 µm. Samples were prepared, and then tested in triplicate at each time point using low-power field (LPF) modes. Particle size was evaluated by calculating the diameter of a circle with the same cross-sectional area as the particle (CE diameter). The shape of particles was determined by their circularity (Σ), which is the ratio between the circumference of a circle with the same area as the projected area of the particle image (S), and the circumference of the particle image (T) (Σ=S/T).

**Raman Microscopy** Raman microscopy was conducted using a Nicolet Almega XR with a 532 nm laser (Thermo Fisher Scientific K.K., Kanagawa, Japan). Microscopic images were obtained by observation of L-ASP aggregate entrapment in the micro-chamber using BX-51 (Olympus Corporation,

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Fig. 3. The Percent Relative Abundance of Total L-ASP (a), L-ASP Monomer (b) and L-ASP Oligomer (c) in Prepared Samples from HP-SEC Analysis

Each value represents the mean±S.D. (n=3). * Significant at p<0.05. ** Significant at p<0.01.
Spectra of observed l-ASP aggregates in the micro-chamber were obtained using 10 scans with a 6s exposure time.

**Statistical Analysis** Particle number data (expressed as the mean±standard deviation (S.D.)) in Figs. 1, 3, 6, and 7 and Table 2 were analyzed by Tukey’s multiple comparison tests with ANOVA. Statistical analyses were carried out using SPSS, and a p-value less than 0.05 was considered significant.

**Results and Discussion**

**Characterization of l-ASP Solution Prepared with Siliconized and Non-siliconized Syringes**

Many studies have shown that various environmental stress factors affect protein stability. Silicone oil, in particular, induced protein aggregation by interacting with protein molecules.\(^{13-18}\) l-ASP was commercialized as a lyophilized formulation in Japan. To investigate the effect of syringe type on the dissolution of lyophilized l-ASP, the lyophilized l-ASP was dissolved with 5% glucose solution in siliconized and non-siliconized syringes. Table 2 summarized percent relative abundance of protein species in l-ASP solution prepared with siliconized and non-siliconized syringes (before shaking sample).

In the l-ASP solution prepared with siliconized syringes, all l-ASP segments were decreased, as compared to the non-siliconized system. These results indicated that l-ASP aggregation occurred during the dissolution process in siliconized syringes.

Since detectable particle images were not observed by FPIA analysis in the l-ASP solutions prepared with siliconized and non-siliconized syringes (data not shown), dissolved solutions were characterized by flow cytometry and far-UV CD spectrometry.

Figure 1 shows the flow cytometry results of l-ASP in 5% glucose solution prepared with siliconized and non-siliconized syringes. A 5% glucose solution was used as a blank sample. In the blank sample, 3.2E+5±2.3E+4 particles/mL were observed. Although both l-ASP solutions were transparent, over 2.0E+7 particles/mL were observed in both solutions. These results indicated that sub-visible l-ASP aggregation occurred in both samples. However, no difference in side scatter distribution of side scatter versus forward scatter distribution was observed in both l-ASP solutions (Figs. 1b, c), indicating that no significant change in submicron range particle characterization was observed in either system.

Figure 2 shows the far-UV CD spectra of l-ASP solution prepared with siliconized (solid line) and non-siliconized syringes (dotted line).
Far-UV CD analysis can provide information about the secondary and tertiary structure of proteins. Torosantucci et al. reported that secondary structural differences between plain and PEGylated-insulin could be characterized by far-UV CD. In this study, slight differences in L-ASP secondary structure were observed in the L-ASP samples prepared with siliconized and non-siliconized syringes. Combined, Figs. 1 and 2 suggest that silicone oil in syringe induced L-ASP aggregation upon the dissolution of lyophilized L-ASP in the soluble region.

**Effect of Preparation Method on the L-ASP Aggregation**

Air and oxygen can also affect protein aggregation. During the preparation of injectable protein drugs, disposable syringes and needles are usually used, which contain silicon oil as a lubricant. Air can be added to the syringe headspace to enhance the mixing efficiency during preparation. Therefore, it is important to determine the effect of silicone oil and headspace air on L-ASP aggregation.

To investigate the effects of silicone oil and air on L-ASP aggregation during the shaking process, four samples were prepared. Figure 3 shows the percent relative abundance of the total, monomer and oligomer, as determined by HP-SEC analysis. The percent relative abundance of the total L-ASP spcies were 89.7±0.3 to 94.6±0.4%. In all samples, 72.5±0.3 to 76.5±0.3% L-ASP monomer and 17.2±0.1–18.0±0.1% oligomer co-existed.

Fig. 6. Microscopic Images of Particles Observed in the Micro-chamber and Their Raman Spectra

A silicone oil droplet observed in the Sample 2(a), Raman spectrum of (a) (b), one of particles containing silicon oil droplets observed in Samples 2(c), Raman spectrum of (c) (d), Raman spectra expanded intensity of (d) (e), an observed particle from a Sample 4(f), Raman spectrum of (f) (g).
As shown in Table 2, all segments were decreased for the comparison of L-ASP solution. The percent relative abundance of all segments in non-siliconized syringe systems is higher than that of all segment in siliconized syringe systems. No significant change in monomer and oligomer content were observed among prepared samples.

Figure 4 shows the particle number in L-ASP solutions prepared by four different methods as measured by FPIA analysis.

Based on the data in Fig. 4, it is evident that silicone oil and headspace air in the syringe (Sample 2) affected particle formation. The particle number was $4.8 \times 10^6 \pm 2.1 \times 10^6$ particles/mL. Figure 5 shows the particle images observed in Sample 2. Many particles with various circularities and diameters were observed in Samples 2, and the low overall circularity of these samples represented the presence of fibrous particles. To characterize these fibrous particles in the micro-chamber, fibrous particles were collected and a Raman microscopy study was performed. The results are shown in Fig. 6.

A high circularity droplet was observed in the micro-chamber (Fig. 6a), and its Raman spectrum is shown in Fig. 6b. Sharp, strong stretching vibrations at 2904 and 2961 cm$^{-1}$

**Fig. 7.** Particle Number and Their Characterization for Samples 1–4 as Measured by Flow Cytometry Analysis

Particle number (/mL) (a), forward scatter vs. side scatter dot plot of Samples 2(b) and 4(c). Each value in Fig. 7(a) represents the mean±S.D. (n=3). *Significant at $p<0.05$. **Significant at $p<0.01$.

**Table 3.** Change of the Particle Number of Aggregated Sample by Storage from FPIA Analysis

| Storage time (h) | Sample 1       | Sample 2       | Sample 3       | Sample 4       |
|-----------------|----------------|----------------|----------------|----------------|
| 0               | $933\pm115$    | $4233\pm30015$ | $466.7\pm115$  | $267\pm115$    |
| 1               | $600\pm400$    | $27800\pm16588$| $333.3\pm115$  | $533\pm306$    |
| 3               | $400\pm200$    | $19533\pm5900$ | $133.3\pm115$  | $267\pm115$    |
| 6               | $1267\pm1102$  | $65067\pm7159$ | $466.7\pm503$  | $333\pm231$    |

Data represents the mean±S.D. (n=3). From the result of FPIA analysis, no significant change of the particle number of aggregated samples by storage were observed.
were assigned to symmetric stretching of CH$_3$ and CH$_2$ in poly(dimethylsiloxane) which is one of components of silicone oil, respectively. Absorption bands at 483 and 703 cm$^{-1}$ were assigned to Si–O symmetric stretching and C–H of CH$_3$ symmetric stretching vibration, due to the silicone oil. These results indicated that the observed droplet was silicone oil, which was present in the preparation devices.

In Fig. 6c, a particle containing a droplet was observed, suggesting that sub-visible particle formation was initiated by the presence of the silicone oil. Raman spectra of the particle in Fig. 6c were taken to confirm that the silicone oil induced sub-visible particle formation. These results indicated that the observed droplet was silicone oil from the preparation devices. A Raman spectrum of the sub-visible particle in Fig. 6c is shown in Fig. 6d, and an expanded spectrum is depicted in Fig. 6e. Several peaks were observed in addition to the silicone oil peaks. Vibrational peaks at 998, 1255, and 1660 cm$^{-1}$ were assigned to phenylalanine, amide III, and amide I, respectively. In the interview form for L-ASP, it was reported that L-ASP exists as a tetramer, and that eight phenylalanine molecules are contained in each monomer. These results indicated that the fibrous products shown were derived from L-ASP. Therefore, it was revealed that the observed aggregate product contained silicone oil, and that silicone oil and headspace air induced L-ASP aggregation during shaking process.

The size of the aggregated proteins varied. The acceptable particle range for FPIA after calibration is approximately 2 to 40 µm. Silicone oil might affect L-ASP molecules in smaller particle ranges (sub-visible and submicron region). To investigate the effect of silicone oil on L-ASP aggregation below the detection limit of FPIA, flow cytometry measurements were performed using Samples 1 to 4. The particle number and forward scatter vs. side scatter plot results are shown in Fig. 7.

The particle number in Samples 1 and 2 were decreased by shaking for the non-shaking sample with siliconized syringe (Fig. 1a). From the comparison of HP-SEC results between before and after shaking Samples 1 and 2, the percent relative abundance of total L-ASP species (%) decreased by shaking (Table 2, Fig. 3a). Since a large number of particles were observed in the FPIA analysis of Sample 2, it was suggested that the particles detected by flow cytometry decreased, leading to larger aggregation products. Side scatter particle distribution was clearly shifted to the higher side scatter values of Sample 4.
by shaking (Figs. 1b, 7b). And shaking process resulted in the wider particle distribution of side scatter. It was reported that side scatter values are proportional to cell granularity or internal complexity. Nishi et al. reported that flow cytometry is available to analyze protein aggregation produced by heat, freeze-thawing, shaking, and light exposure. Particle distributions were changed by these environmental stressors. Therefore, these results indicated that interaction of L-ASP with silicone oil promoted protein aggregation, which resulted in the higher side scatter distribution.

As shown in Fig. 7, particle number in Samples 3 and 4 was increased by shaking (Fig. 1a: non-siliconized syringe system). The particle distribution did not change before and after shaking using non-siliconized syringes (Figs. 1c, 7c). Since small amount of aggregated particles were observed before and after shaking in non-siliconized syringes, these results indicated that aggregated proteins might be dispersed by shaking with headspace air in non-siliconized syringes.

The FPIA and flow cytometry measurements strongly suggested that silicone oil interacted with L-ASP molecules in the sub-visible and submicron regions. Protein aggregation occurred after initiation by silicone oil during preparation with siliconized syringes using shaking and headspace air. Many authors reported that air–water interfaces affect the protein stability. Our data suggested that the presence of air during shaking increased the air–water interface in the syringe, thereby enhancing the mixing efficiency.

Change in Aggregated L-ASP Particle during Storage

From the interview form on L-ASP, it is recommended that L-ASP injection should be administrated after preparation for formation of protein aggregation. As mentioned in introduction, prepared injections might be frequently stored for several hours prior to injection in actual clinical situation of Japan. In the case of L-ASP injection for intramuscular or subcutaneous use, prepared L-ASP in 5% glucose solution is frequently stored in the syringe until administration. Since there is silicone oil in the siliconized syringe, characterization of prepared L-ASP injections in the syringe is important. To investigate the effect of storage on protein aggregation, stability tests were performed for samples prepared using the four methods. The results of FPIA analysis to investigate the protein aggregation in micro order particles are shown in Table 3.

A large number of particles were observed in Sample 2, and no significant change in the number of aggregate particles was observed with increased storage time. In Samples 1, 3, and 4, a small number of particles was observed, although the number of particles was not significantly changed by storage time. These results clearly indicated that the effects of silicone oil and air during preparation did not change with storage in a micro order range.

To investigate the effect of silicone oil on L-ASP aggregation below the detection limit of FPIA, the change in particle number as a function of storage time was examined by flow cytometry (submicron region). These results are shown in Fig. 8.

These results clearly indicated that silicone oil affects L-ASP aggregation in sub-visible and submicron regions.

That is, no significant change in particle number was observed with increased storage time in Samples 1 and 2 (Figs. 8a, b). On the other hand, the particle number decreased with storage time in Samples 3 and 4. As we mentioned in the previous section, forward scatter versus side scatter dot plots of the L-ASP solutions prepared with non-siliconized syringes did not change by shaking (Figs. 1c, 7c). Thus, the detected particles in sample 4 likely did not include silicone oil, based on the results of Raman microscopy (Figs. 6f, g). Therefore, the detected particles in the non-siliconized system could be soluble aggregated proteins that dispersed with increased storage time. These results indicated that particle characterization of samples prepared with siliconized and non-siliconized syringes differed in the sub-visible, especially submicron region, and that silicone oil strongly affects the stability of aggregate products.

Conclusion

Highly concentrated solutions were prepared from lyophilized L-ASP with siliconized and non-siliconized syringes. Silicone oil in the syringe and the presence of air during shaking strongly affected the aggregation of L-ASP; based on the results of FPIA and flow cytometry analyses. L-ASP aggregates containing silicone oil were stable for the 24 h after preparation. Therefore, excessive shaking should be avoided when preparing L-ASP injections in siliconized syringes. Although the use of non-siliconized syringes is not widespread in clinics, preparation of L-ASP with non-siliconized syringes is one of a good choice to reduce L-ASP aggregation.
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