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IIII Review IIII

JCB-SGT Crystallization Devices Applicable to PCG Experiments and their Crystallization Conditions

Sachiko TAKAHASHI[1], Misako KOGA[1], Bin YAN[1], Naoki FURUBAYASHI[2], Masayuki KAMO[2], Koji INAKA[2] and Hiroaki TANAKA[1]

Abstract

JCB-SGT, which is the primary crystallization container used in JAXA PCG (Protein Crystal Growth) experiments, is simple, versatile and has a wide range of applications. Various crystallization methods can be applied in JCB-SGT: counter-diffusion method, dialysis method, vapor-diffusion method, osmosis-tube method and batch method. We have supported users of JAXA PCG for nearly 20 years and incrementally improved our technologies to obtain high-quality protein crystals in space. In this review, the outline of JCB-SGT is described and important insights and lessons learned from our experiences in optimizing crystallization conditions are explained. These are helpful for PCG users to optimize their protein samples for experimentation, contributing to both space-based experiments and also to laboratory experiments on the ground.

Keyword(s): Protein crystallization, JCB-SGT, Crystallization device, Counter-diffusion, Vapor-diffusion, Batch, Dialysis, Osmosis, Space experiment, Microgravity, JAXA
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1. Introduction

NASDA (National Space Development Agency of Japan, the predecessor of the Japan Aerospace Exploration Agency [JAXA]) started protein crystallization experiments in space in 1992 using an original batch device (STS-47 (FMPT)) [1]. After this start, NASDA launched protein samples in 1997 (STS-84) and 2003 (STS-107) using CVDA [2] and HDPCG [3], respectively. Those two devises were provided by the UAB (University of Alabama Birmingham) [4]. In parallel to this, NASDA initiated regular flight opportunities by using Russian vehicles such as the Soyuz and Progress (NASDA GCF) [5], by using Granada Crystallization Box (GCB) [6]. GCB was developed by Garcia-Ruiz's group, was easy to use and low-cost. We have technically supported NASDA/JAXA since then [7, 8]. We have incrementally improved the original GCB concept to the present device, the JCB-SGT (JAXA Crystallization Box – Sealbag Gel-tube). In this review, an outline of JCB-SGT and methods to use it are explained.

2. Configuration of Crystallization Device

Current JAXA PCG project uses both Russian flights (20°C) and United States (US) / Japanese (JPN) flights (4°C and 20°C) to transport JCB-SGT to the International Space Station (ISS). In both cases, JCB-SGT is used as the standard container for crystallization cells.

2.1 Russian Flight

The configuration "layers" of JCB-SGT containers for experiments conducted using Russian flights to the ISS are shown in **Fig. 1(A)**. In Russian flights, since the crystallization experiment is completely passive after sample loading, crystallization conditions are optimized to start crystal growth in orbit. Otherwise crystals may start growing before launch because the diffusion process for crystallization start just after the samples are loaded.

2.2 US/JPN Flight

The configuration "layers" of JCB-SGT containers for experiments conducted using US or Japanese flights to the ISS (Falcon by SpaceX or HII B by JAXA) are shown in **Fig. 1(B)**. To inhibit crystal growth prior to reaching the ISS, the gel-tube attached to the capillary is compressed to stop diffusion by the JCB-SGT suppression mechanism (JCB-SGT PRESS) until they are delivered to the ISS and released by astronauts.

3. JCB-SGT

JCB-SGT is a transparent container made of polyethylene terephthalate (PET) sheet, TECHBARRIER™ (Mitsubishi Chemical) (**Fig. 2(a)(b)**). Since it is resistant to gas permeation, the solution in the container can be preserved for years. In each cell of the JCB-SGT three-cell type **(Fig. 2(a))**, the basic configuration is for 1 ml of reservoir solution and one or two capillaries to be installed (**Fig. 2(f)**). In each cell of the JCB-SGT six-cell type (**Fig. 2(b)**), the basic configuration consists of 0.5 ml of reservoir solution and one capillary (**Fig. 2(g)**). JCB-SGT six-cell type is used when different crystallization condition is applied for each capillary.

1 Confocal Science Inc. 7F Hayakawa 2nd Bldg, 2-12-2 Iwamoto-cho, Chiyoda-ku, Tokyo 101-0032 Japan.
2 Maruwa Foods and Biosciences Inc. 170-1 Tsutsui-cho, Yamatokoriyama, Nara 639-1123 Japan.
(E-mail: tanakah@confsci.co.jp)

A derivative is the JCB-SGT DX, one side of which is made of an oxygen absorbing sheet, AGELESS OMAC (Mitsubishi Gas Chemical) (**Fig. 2(c)(d)**). This is designed for use with protein samples which should be crystallized under deoxidized condition. In each cell of JCB-SGT DX 3-cell type (**Fig. 2(c)**) and JCB-SGT DX 2-cell type (**Fig. 2(d)**), 1 ml of reservoir solution and two capillaries (outer diameter (OD) 1.2 mm) are loaded.

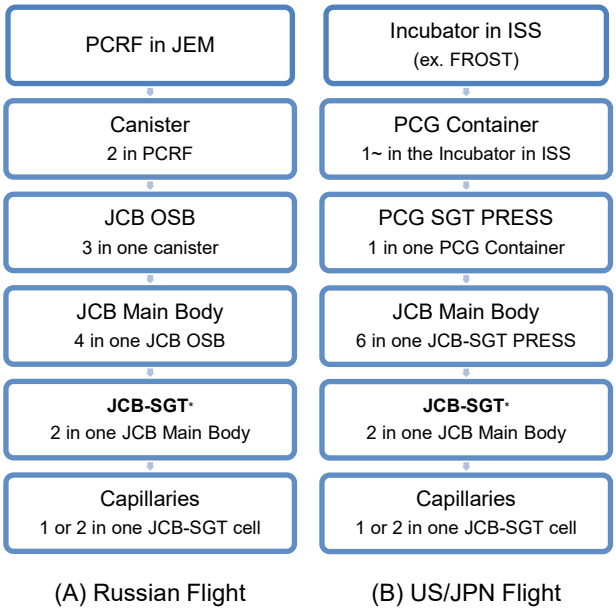

Fig. 1 Configuration layer of crystallization device for JAXA PCG. *: Four types of JCB-SGT: JCB-SGT 3-cell, 6-cell type, JCB-SGT DX 2-cell and 3-cell type.

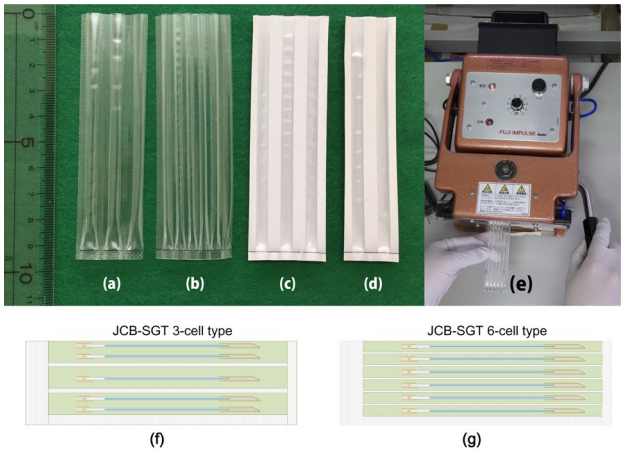

Fig. 2 JCB-SGT. (a) JCB-SGT 3-cell type, (b) JCB-SGT 6-cell type, (c) JCB-SGT DX 3-cell type, and (d) JCB-SGT 2-cell type. (e) Capillaries are installed in JCB-SGT and JCB-SGT is sealed. (f) In JCB-SGT 3-cell type, basic configuration is for 1 ml of reservoir solution and one or two capillaries to be installed. (g) In JCB-SGT 6-cell type, basic configuration is for 0.5 ml of reservoir solution and one capillary to be installed.

For Russian flights, two JCB-SGT / -SGT DX are set in the JCB Main Body which is a small flat square container with a lid made of acrylonitrile - butadiene - styrene (ABS) resin (35*100*5mm). Four JCB Main Bodies are grouped to compose JCB OSB (Outer Sealing Bag) (**Fig. 1(A)**).

For US / JPN flights, JCB-SGT PRESS is provided to stop crystallization before reaching orbit. Two JCB-SGT / -SGT DX are set in JCB Main Body with a pressing plate on which the gel-tube part of capillaries (**Fig. 3**) are placed. Six JCB Main Bodies are grouped and compressed by JCB-SGT PRESS with a screw (**Fig. 1(B)**). After arrival at the ISS, an astronaut releases the screw and diffusion starts.

4. Crystallization method

Multiple crystallization methods can be applied in JCB-SGT: counter-diffusion (CD) method, dialysis (DL) method, vapor-diffusion (VD) method, osmosis tube (OT) method, and batch method. Based on the original capillary configuration of CD method (**Fig. 3**), we derived capillaries for other crystallization methods using a similar configuration. It is possible to vary the diameter of the capillary according to the target size of crystals to be grown. Feature of each method is described in section 6.

4.1 Counter-diffusion method

The CD method is a crystallization method using diffusion [9]. The original CD method, GCB [6], was developed by Garcia-Ruiz, CSIC-University of Granada and used for the initial NASDA-GCF project. As an enhancement to GCB, we developed the Gel-tube (GT) method [10]. Currently, most protein samples in JCB-SGT use the CD method with GT. The crystallization capillary consists of a glass capillary in which the protein solution is loaded and a gel-tube composed of agarose gel in silicone tubing, which is attached as a diffusion barrier at the capillary end (**Fig. 3**).

The reservoir solution components diffuse into the capillary

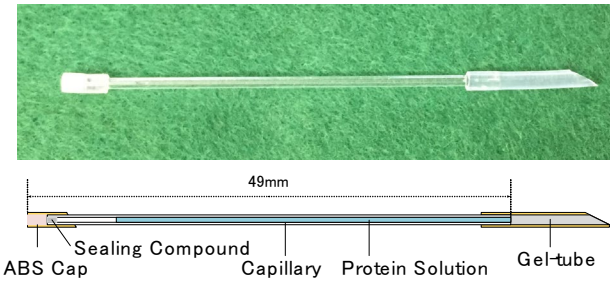

Fig. 3 Counter-diffusion (CD) method. A straight capillary (47mm in length and bore in 0.3mm, 0.5mm or 0.7mm) is filled with protein solution (40mm in length). A piece of gel-tube (6~15mm in length) is attached to the end of the capillary. The other end of the capillary is sealed with a sealing compound. An ABS cap is applied to prevent leakage of the solution caused by osmotic pressure over long periods of time.

via the gel-tube. Conversely, the protein sample and other coexisting components in the capillary diffuse out of the capillary through the gel-tube. As a result of such bi-directional diffusion, a broad range of concentrations of the reservoir solution and protein sample are scanned, so that various combinations of protein sample concentration and reservoir solution concentration are achieved in the capillary. This process is called a CD method since it uses such diffusion [9].

4.2 Dialysis Method

The DL method is a method of diffusing a reservoir solution into a protein sample via a dialysis membrane. In general, dialysis buttons [11, 12] for microdialysis method are commercially available for crystallization. But to fit in JCB-SGT, we developed a dialysis method using a capillary as shown in **Fig. 4**. Unlike the dialysis button, in our DL method the diffusion path is long and the osmotic pressure difference is not great, so it is not likely that bubbles will be generated within the internal solution of dialysis.

Attaching a small piece of dialysis membrane between the capillary end and the gel-tube top is not easy, so we use C-Chip DM which is a combination of dialysis membrane and gel-tube (see in section 5.2).

The diffusion profile is different from the CD method (a detailed explanation is given in section 6.3).

4.3 Vapor-Diffusion Method

The standard VD method is one in which a protein sample and a reservoir solution are mixed (crystallization drop) and a reservoir solution interacts with the drop via an air layer [13]. Various containers are commercially available for crystallization by the VD method.

Here we introduce the VD method as used in JCB-SGT (**Fig. 5**). A solution is prepared by mixing the protein sample and reservoir solution (protein/reservoir mixture), usually in a ratio of 1:1, which is loaded in a capillary. A few millimeters of air gap is placed in the capillary end and a gel-tube is attached at the capillary end. The gel-tube is then placed in the reservoir solution. The protein/reservoir mixture is concentrated by the osmotic pressure difference with the reservoir solution in the gel-tube via the air gap. As a result, all the components in the protein/reservoir mixture are gradually concentrated and crystals start growing when the concentration of the components reaches the necessary concentration.

In space experiments, the VD method is said to cause Marangoni convection when moisture evaporates from the surface of the crystallization drop [14, 15]. It induces flows in drops which disturbs the suppressed convection which is an expected feature of the microgravity environment. Therefore, it was pointed out that the use of VD method in space experiment should be avoided [15]. However, in the VD method using the capillary, it is considered that the Marangoni convection in the reservoir solution can be decreased since the surface area of the liquid phase is much smaller than the crystallization drop in conventional vapor-diffusion cells.

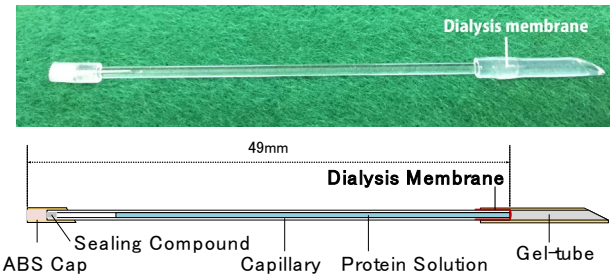

Fig. 4 Dialysis (DL) method. The configuration is almost same as CD method except for a small piece of dialysis membrane inserted between the capillary end and the gel-tube top.

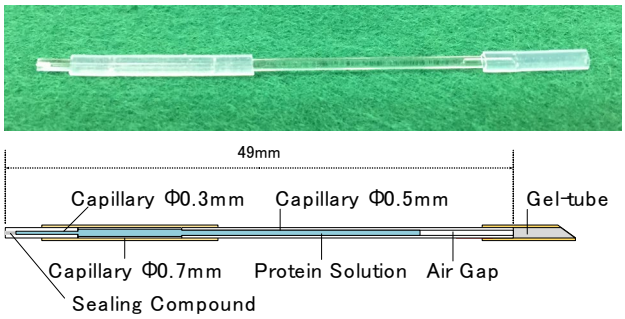

Fig. 5 Vapor-diffusion (VD) method. The crystallization capillary, which is made of three pieces of glass capillary (bore of 0.3mm (7mm in length), 0.7mm (10mm in length) and 0.5mm (32mm in length)) and a piece of silicone tubing, is filled with protein solution from the capillary of 0.3mm bore, leaving an air gap. A piece of gel-tube (6~15mm in length) is attached to the end of the capillary. The other end of the capillary is sealed with a sealing compound.

4.4 Osmosis-Tube Method

The OT method is one in which a protein sample and a reservoir solution are mixed 1:1 and interact with a reservoir solution via a water permeable membrane, originally developed by Yamada et al [16]. They used a urethane membrane as the permeable membrane; but we developed a simpler structure one, which is scalable to other JCB-SGT crystallization capillaries, using silicone tubing as a water permeable membrane (**Fig. 6**).

In the OT method, a solution prepared by mixing a protein sample and a reservoir solution in a ratio of 1:1 (protein/reservoir mixture) is loaded in a capillary equipped with silicone tubing at one tip. At the other tip, is an air gap of a few millimeters-length. The gel-tube is attached and the entire capillary is placed in the reservoir solution. The gel-tube is attached via the air gap in order to compensate for negative pressure in the capillary by water permeation through the silicone tubing.

The water of the protein/reservoir mixture is transferred to the reservoir solution side by the osmotic pressure difference with the reservoir solution outside the silicone tubing. As a result, all the components in the protein/reservoir mixture are gradually concentrated and crystals start growing when concentrations in the protein/reservoir mixture reach those at which crystals grow.

The crystallization capillary of VD method (**Fig. 5**) and OT method (**Fig. 6**) look very similar. A difference between them is that, in VD method, the protein solution is all in a glass capillary, but, in OT method, a part of the crystallization capillary is made of silicone tubing which has an osmotic role.

4.5 Batch Method

The batch configuration is simple. A mixture of the protein sample and the reservoir solution is loaded into the capillary and the both ends of the capillary are sealed by sealing compound and the ABS cap. The diameter of the capillary can be varied depending on the size of crystals to be grown. The configuration of the device is shown in **Fig. 7**.

5. Components of Crystallization Capillary

5.1 Capillary

All the capillaries described below fit in JCB-SGT and have application to JAXA PCG.

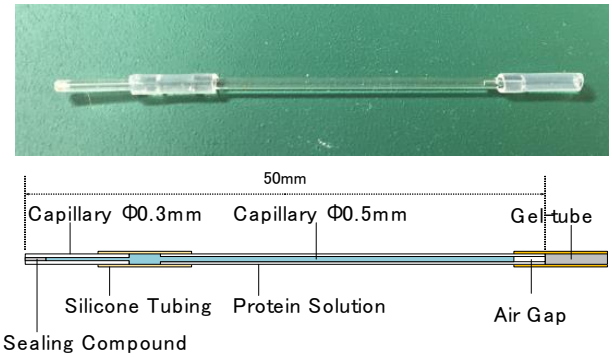

Fig. 6 Osmosis tube (OT) method. The configuration is almost same as VD method except that, in OT method, the protein solution is exposed to the silicon tubing.

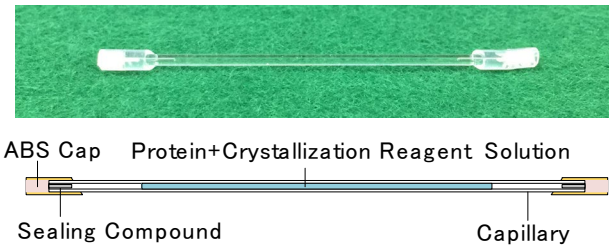

Fig. 7 Batch method. A straight capillary (any length and any bore size) is filled with protein and reservoir solution mixture. Both ends of the capillary are sealed with a sealing compound and the ABS cap.

5.1.1 Straight Capillary (SC)

The standard capillary that can be used in JCB-SGT is a straight tube of Duran glass with an OD of 1.2 mm and a length of 47 mm. There are three types of bore: 0.3 mm, 0.5 mm and 0.7 mm. The sample is usually filled to 40 mm length, with a sample volume of 2.8, 7.9 and 15.4 µL in each type of bore, respectively.

5.1.2 Large Bore Capillary (LC)

The largest bore of the standard SC is 0.7 mm. To obtain crystals larger than this, a larger bore capillary is required. When large crystals are desired, it is preferable that the number of crystals be as small as possible, preferably only one. To do this, the capillary opening should be smaller to reduce nucleation probability. Based on these requirements we developed a capillary of 1.95 mm bore (with a volume of 28.3 µL) to which a capillary of 0.3, 0.5 or 0.7 mm bore is connected (**Fig. 8**). A crystal of large PcCel6A grown in LC is shown in **Fig. 9**. The protein solution was 40 mg/ml PcCel6A, 5 % polyethylene glycol (PEG) 4000, 80 mM NaCl, 10 mM cellotriose in 50mM sodium acetate buffer pH 5.5; the reservoir solution was 47.5% PEG 4000, 5 mM cellotriose in 50mM sodium acetate buffer pH 5.5. The crystal started growing five weeks after crystallization set-up and grew to 1 mm length after eight weeks.

The LC on which a dialysis membrane is attached to the capillary connection part is LCDM (**Fig. 10**). When growing large crystals the LCDM is helpful in cases with small protein

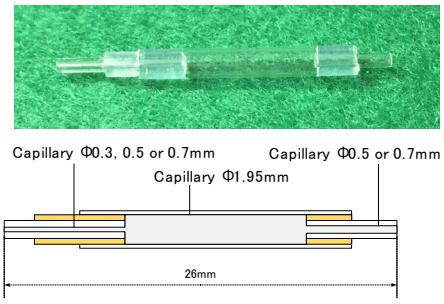

Fig. 8 Large bore capillary (LC). The crystallization capillary, which is made of three pieces of glass capillary (bore of 0.3, 0.5 or 0.7mm (8mm in length), 1.95mm (18mm in length) and 0.5 or 0.7mm (6mm in length)).

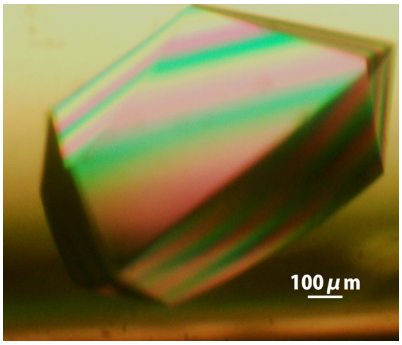

Fig. 9 A large crystal of PcCel6A grown in LC.

molecules and a long time for crystallization because the amount of protein which diffuses out of LC through the gel-tube may be substantial. Especially for growing large crystals, losing protein is a serious problem. To solve this we carefully attach a piece of dialysis membrane between the large bore capillary and the thin capillary, which makes the reservoir solution diffuse into the protein solution but the protein stays in the capillary.

There is also be the problem that crystallization frequently occurs in the thin capillary of the LC capillary by the CD method so that large crystals cannot be obtained in some cases. Using LCDM, crystallization in the thin capillary can be avoided. The LCDM also has a content volume of 28.3 µL. **Figure 11** shows a large crystal of lysozyme grown in LCDM. The protein solution was 45 mg/ml lysozyme, 5 % PEG 4000 in 50mM sodium acetate buffer pH 4.5; the reservoir solution was 5 % PEG 4000, 700 mM NaCl in 50 mM sodium acetate buffer pH 4.5. The crystal started growing three days after crystallization set-up and grew to 1 mm length in five weeks.

5.1.3 Diffusion Pair

Usually the reservoir solution is fully loaded in JCB-SGT cells in which SC, LC or LCDM is placed. However, when it is desired to reduce the reservoir volume, such as when expensive ligands are used in the reservoir solution, the reservoir solution is placed in the LC and connected to the SC or the LC via the gel-tube to form a diffusion-pair-type crystallization configuration (**Fig. 12**), which is loaded directly into the JCB-SGT 6-cell type.

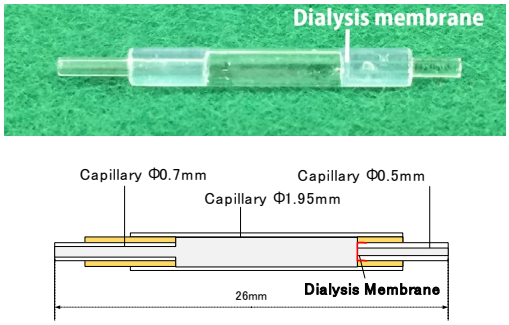

Fig. 10 Large bore capillary with dialysis membrane (LCDM). The crystallization capillary, which is made of three pieces of glass capillary (bore of 0.7mm (8mm in length), 1.95mm (18mm in length) and 0.5mm (6mm in length)). A small piece of 'built-in' dialysis membrane is placed in the capillary connection part.

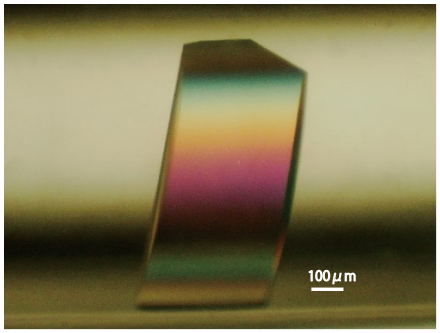

Fig. 11 A large crystal of lysozyme grown in LCDM.

5.2 Gel-Tube (GT)

A standard type gel-tube is silicone tubing (Silascon Medical Tube, Kaneka Medix Corporation) with a bore of 1 mm and OD of 2 mm filled with agarose gel.

A derivative of GT is C-Chip DM (Confocal Science Inc.) (**Fig. 13**) which is designed for attaching a dialysis membrane easily between the capillary and the gel-tube. The protein sample is filled in a capillary and the C-Chip DM is attached to the capillary end so that the dialysis membrane adheres closely to the capillary end.

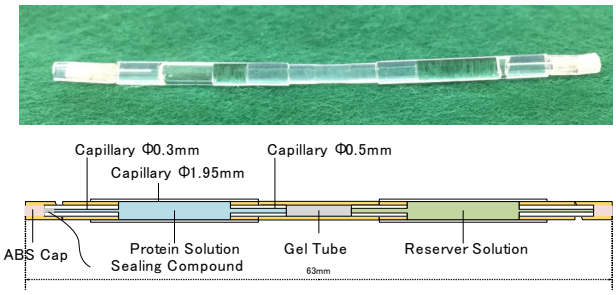

Fig. 12 Diffusion pair. LC and LC are connected via the gel-tube. The protein solution is loaded in one LC and the reservoir solution is loaded in the other LC.

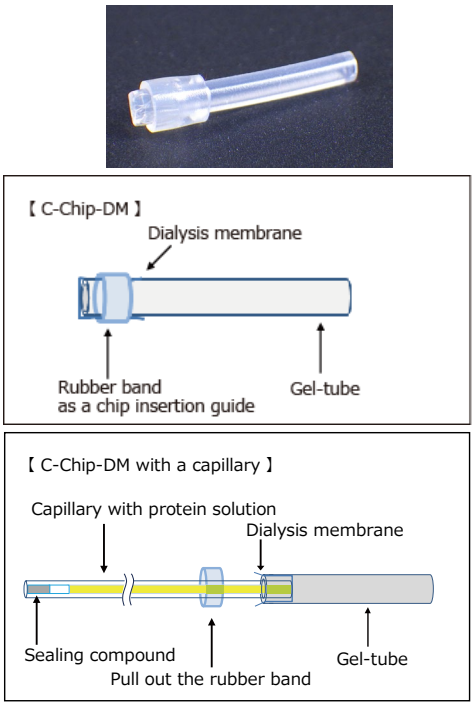

Fig. 13 C-Chip DM. C-Chip DM with gel-tube can be easily attached to the capillary using the attached rubber band.

5.3 Sealing Compound and ABS Cap

The end of the capillary is usually sealed by inserting a sealing compound (Fisherbrand Hemato-Seal) into the capillary. However, this method is incomplete under the condition that high osmotic pressure is applied over a long period (several weeks or more). When placed under such conditions, it is better sealed by attaching a cap with ABS resin to the capillary to avoid leakage of the solution caused by osmotic pressure (**Fig. 14**).

6. Crystallization Condition

It is necessary to optimize crystallization conditions according to the characteristics of each container and method. It is thought that crystallization of proteins can be caused by mixing protein samples and major reservoir solution to establish concentration conditions to grow crystals. In practice, not only the major crystallization reagents but also minor reagents, that is, salts, metals, single- and multi-valent organic acids, single- and multi-valent bases and organic solvents affect crystallization [11, 12, 17]. The effect of these reagents is usually understood using a phase diagram with the concentrations of these components as coordinate axes.

In the batch method, the initial crystallization condition is one point on the phase diagram and will not change before initiation of crystallization. In other methods, the condition varies with time on the phase diagram, so it is necessary to understand the time course for each method, as described below.

6.1 VD and OT methods

In VD and OT methods, a solution of a protein sample and a reservoir solution (usually mixed in a ratio of 1:1 protein/reservoir) and a reservoir solution interact via a gas layer or a permeable membrane.

The protein/reservoir mixture contains protein and other components coexisting with the protein sample, as well as the crystallization reagent and its coexisting components derived from the reservoir solution. By 1:1 mixing, the concentrations of every component in the protein/reservoir mixture are halved. Water diffuses due to the interaction with the reservoir and the mixed solution, causing the mixture to concentrate [13].

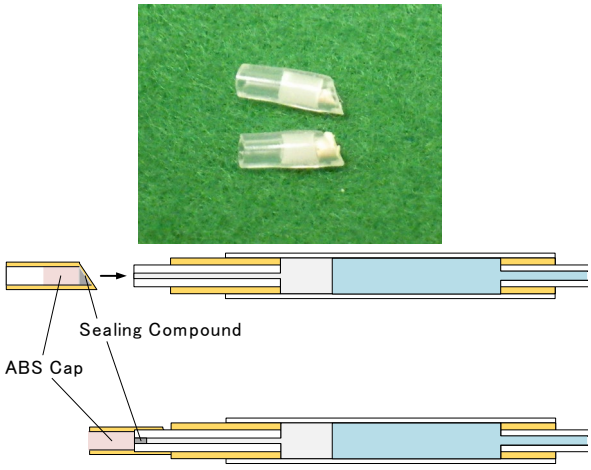

Fig. 14 ABS-Cap. Sealing the end of the capillary with ABS-Cap can avoid leakage of the solution caused by osmotic pressure.

As a result, all concentrations increase towards the original reservoir concentration. Crystals are formed when the concentration levels reach a sufficient level.

In many cases, it is more likely that crystals will grow if the concentration of the components becomes higher at the same time. Thus, VD method can be crystallized easier than other methods.

However, with this method, it is not possible to control the concentration levels of the components individually. There are some cases in which crystals cannot be grown for this reason (for example, when other components coexisting in the protein sample are not favorable for crystallization when they are concentrated).

The VD method is a method used by many researchers. A large number of screening kits for searching for suitable crystallization conditions are provided commercially, but careful consideration of crystallization conditions is required from the point of view that all components, even coexisting components in the protein sample, are concentrated.

6.2 CD method

In the CD method, generally a protein sample is loaded in a capillary and the reservoir solution components diffuse from the capillary end. At the same time the protein sample and other coexisting components in the capillary diffuse outside the capillary. Therefore, the coexisting components in the capillary are replaced by the components in the reservoir solution.

As a result of such bidirectional diffusion, a combination of wide concentration regions of the crystallization reagent and the protein sample is scanned. But the time profile is not easy to measure. Therefore, we prepared a 1-dimensional (1-D) diffusion simulation program [10] so that various concentrations of components in the capillary can be estimated (**Fig. 15**). It is necessary to study the crystallization condition considering this diffusion time course and the starting condition of crystallization on the phase diagram.

In CD method, components with lower molecular weights diffuse faster than those with high molecular weights like proteins. The low molecular weight components coexisting with the protein sample in the capillary diffuse faster than the protein sample and leak out of the capillary. On the other hand, the main crystallization reagent and the coexisting components in the reservoir solution diffuse into the capillary (**Fig. 16(A)**). In the CD method, it is usual that the volume of the reservoir is much larger than that of the capillary, so that components other than the protein sample in the capillary are mostly replaced with those in reservoir solution. Therefore, the concentration of each

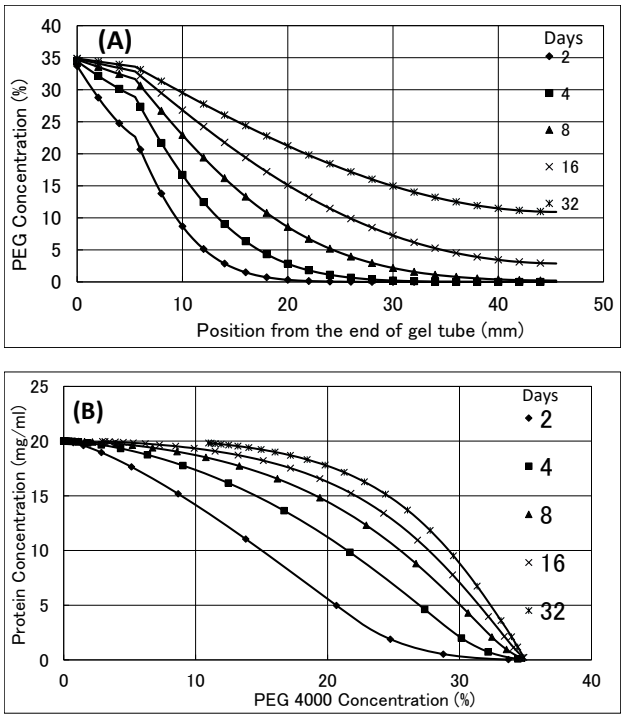

Fig. 15 Concentration profile in a capillary in CD method by 1-D diffusion simulation. The length of the protein solution in the capillary was 40mm, and a 6 mm-length gel-tube was attached to the end of the capillary. (A) shows the diffusion profile of PEG 4000 from reservoir solution into the capillary. (B) shows the PEG 4000 vs. protein concentration relation along the capillary and gel-tube.

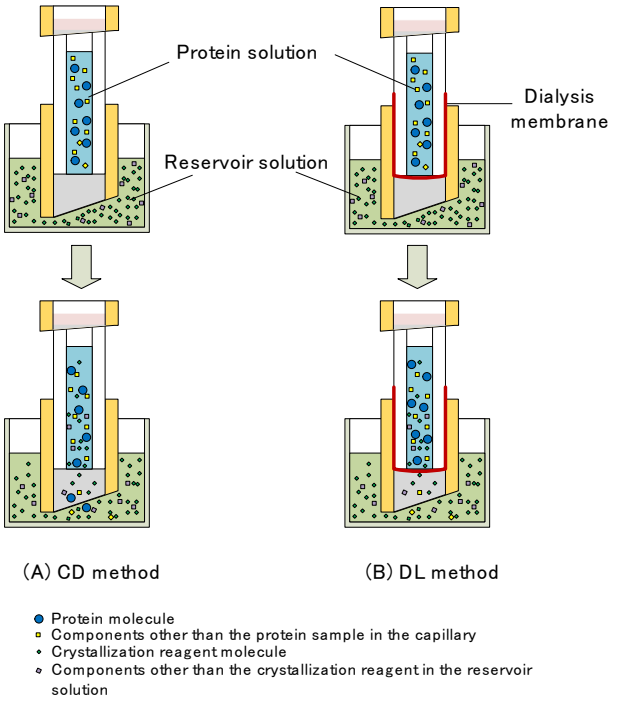

Fig. 16 Diffusion in CD method and DL method. (A) In CD method, the reservoir solution components diffuse into the capillary via the gel-tube. Conversely, the protein sample and other coexisting components in the capillary diffuse out of the capillary through the gel-tube. (B) In DL method, the reservoir solution components diffuse into the capillary via the gel-tube. Conversely, the coexisting components other than the protein molecules in the capillary diffuse out of the capillary through the gel-tube.

component related to the crystallization conditions can be individually controlled and more sophisticated crystallization conditions can be set than with the VD method. For example, in cases where crystals cannot be obtained by the VD method, such as when other components coexisting in a protein sample are not favorable for crystallization, it is often experienced that good crystals can be obtained by the CD method rather than the VD method.

Incidentally, diffusion of protein molecules is greatly reduced in high molecular weight PEG, whereas diffusion of low molecular weight compounds does not slowdown in PEG. Therefore, when a PEG-type crystallization reagent is applied to the CD method, diffusion leakage of proteins can be suppressed, which is preferable.

Presently not many researchers use the CD method. However, if the mechanism as described above is better understood when setting conditions, more optimal crystallization conditions can be established.

6.3 DL method

In the DL method, the diffusion leakage of the protein sample is suppressed by the dialysis membrane attached to the open end of the capillary. As a result, the protein sample does not diffuse or leak, so it will not scan on the phase diagram.

However, among the other components coexisting in the protein sample, a component having a low molecular weight diffuses and leaks out of the capillary. And the main crystallization reagent component and the coexisting components of the reservoir diffuse into the capillary (**Fig. 16(B)**).

Since the reservoir capacity is usually much larger than the capillary content in the DL method, components other than the protein sample in the capillary are replaced with reservoir components as in the CD method. Therefore, the concentration of the reagent component related to the crystallization condition can be individually controlled and similar to the CD method, more advanced crystallization conditions can be set.

Not many researchers use the DL method. However, if the above mechanism is better understood when setting conditions, similar to the CD method, it is a good way to establish more optimal crystallization conditions.

6.4 Diffusion pair

Crystallization with a diffusion pair is not a common method,

but it is effective when it is desired to reduce the amount of the reservoir solution in order to establish a gentler diffusion time course or to add expensive ligands.

One of two capillaries with limited capacity is filled with a protein sample and the other is filled with a reservoir solution and diffused mutually (**Fig. 12**). Since the capacity is limited, the time course of both components must be estimated by 1-D diffusion simulation and the conditions should be optimized (**Fig. 17**).

6.5 For Large Crystal

Obtaining crystals exceeding 1 mm^3 that can be used for neutron diffraction requires that the probability of nucleation in a container be reduced so that only one crystal is grown. For this purpose, a method of gradually increasing the concentration of the reservoir solution to a high concentration protein sample is generally used.

In this case, if the concentration of the crystallization reagent and the concentration of the coexisting components affecting nucleation can be individually controlled, it is possible to more reliably obtain only one large crystal. In this respect, CD method, DL method, and diffusion pair have advantages.

6.6 Enhancing Nucleation

In our many experiences for nearly 20 years, the mixing of the protein sample solutions and reservoir solutions, like in the VD method, has the effect of accelerating nucleation when they are brought into contact at high concentrations. For this reason, we enhance nucleation by mixing the protein sample and reservoir solution at an appropriate concentration ratio for samples in which nucleation is difficult to occur.

In addition, in the case of a sample in which nucleation is still unlikely to occur, a seeding method can also be adopted. A seed solution is added to the solution in which a protein and a reservoir solution are mixed in a ratio at which the seed does not melt nor grow.

In another seeding method, two-step filling to a capillary can be applied. That is, a protein sample solution is loaded first and then a small amount of seed solution is loaded. In the case of LC capillary, a seed solution is loaded in an inlet capillary separated with an air gap (**Fig. 18**). The seed solution moves to the main LC container by osmotic effect and seeding is performed after reaching orbit [18].

7. Conclusion

For space experiments we have developed the JCB-SGT containers and technologies that can accommodate various crystallization methods and have learned many lessons about optimization of crystallization conditions for these methods. As a result our understanding of the effects of crystallization reagents and other components on protein crystallization has deepened and we are making contributions not only to space experiments, but also to crystallization of protein samples on the ground.

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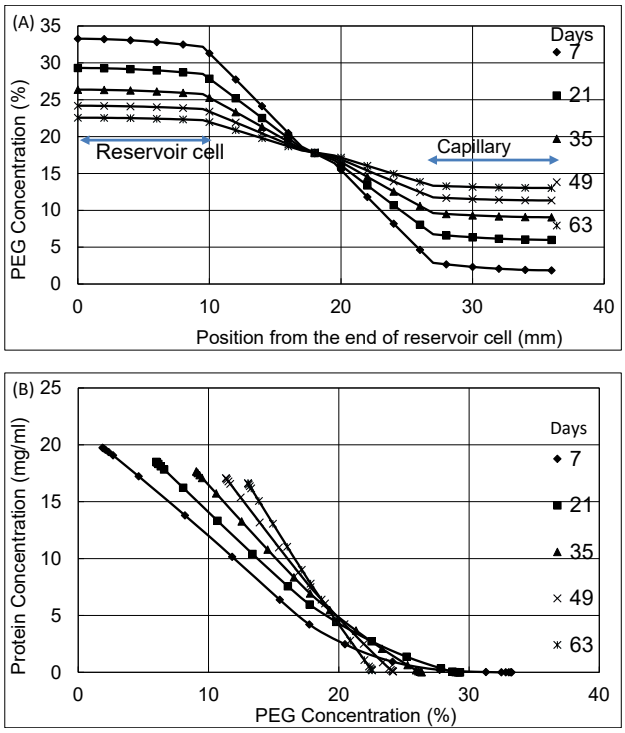

Fig. 17 Concentration profile in a capillary in diffusion pair (LC-LC) configuration by 1-dimensional diffusion simulation. The length of the protein solution and the reservoir solution in LC was both 10 mm, and a 3 mm-length gel-tube was attached in between. 35% PEG 4000 in the reservoir solution diffuses into the protein solution (A). (B) shows the PEG 4000 vs. protein concentration relation along the LC-LC.

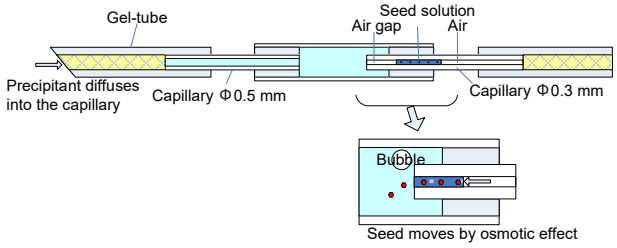

Fig. 18 Seeding in LC method. The seed solution moves to the main LC container by osmotic effect.

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