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# Technical note: an economical apparatus for the observation and harvest of mineral precipitation experiments with light microscopy

**Chris H. Crosby and Jake V. Bailey**

Department of Earth Sciences, University of Minnesota–Twin Cities, Minneapolis, 55455, USA

*Correspondence to:* Chris H. Crosby (crosb118@umn.edu)

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**Abstract.** We describe a small-scale, reusable, and low-cost double-diffusion setup that allows microscopic observation over time for use in mineral precipitation experiments that use organic polymers as a matrix. The setup uniquely accommodates changes in solution chemistry during the course of an experiment and facilitates easy harvesting of the precipitates for subsequent analysis.

## 1 Introduction

Investigations into the influence of organic materials and microbes on authigenic mineral precipitation has transformed our understanding of geosphere–biosphere interactions and improved our understanding of taphonomic processes that allow for the preservation of biological remains. The ability to observe nucleation, precipitation, and growth over time can provide insight into these processes. However, observation and imaging over the course of an experiment, as well as post-experimental analysis, place strict requirements on the experimental setup, including the following:

1. The setup must provide an approaching flux of counterions while simultaneously and sufficiently slowing diffusion to avoid the instantaneous precipitation that would inhibit further crystal growth.

2. To enable microscopic observation over time, the setup must fit a microscope stage during the course of an experiment. The diffusion gel within which precipitation proceeds must be transparent to the imaging wavelength, and for undistorted optical imaging the region and/or material of interest should be housed within a planar (not tubular) transparent housing.

3. For post-experimental analysis of precipitates, the setup must allow harvesting of the materials of interest, which may be both precipitates and various nucleation substrates of interest.

4. The setup should be amenable to a variety of ambient conditions such as temperature, redox conditions, and chemistry. Additionally, the ability to change experimental conditions mid-experiment should allow exploration of increasingly refined and focused questions.

Here, we describe a reusable, small-scale, and low-cost double-diffusion (DD) apparatus that satisfies these requirements and requires only small diffusion gel volumes – a significant advantage when the gel material is expensive or consists of low-volume microbially produced polymeric substances. The apparatus allows detailed observation of progressive precipitation in situ, an example of which can be seen in Fig. S2 of the Supplement.

## 2 Background

A variety of physical setups have been used for decades by chemists investigating mineral precipitation kinetics and are generally one of three types: the single-diffusion (SD) method, in which an ion-containing gel is overlain with a solution of counterions that diffuse into the gel; the double-diffusion (DD) method, in which solutions of constituent ions are separated by a diffusion gel and into which the

ions pass and ultimately meet (Becker et al., 2003); and the constant-composition (CC) method (Morse, 1974; Tomson and Nancollas, 1978). As the name indicates, the CC method holds the ionic strength of constituent ions constant and allows sensitive observation of the impact of factors other than ionic strength. However, for exploring systems relevant to essentially confined environmental systems – such as sediment pores or spaces constrained within polymeric matrices such as those found in sediment or under stromatolitic growth conditions – a diffusion setup is arguably more likely to reflect dynamic in situ conditions, where precipitation leads to falling ion concentrations over time. Hence, diffusion setups are suitable for, and have been used in, studies of biologically mediated precipitation (Becker et al., 2003; Emerson et al., 1994; Hunter et al., 1985).

The DD setup described herein is the result of many iterations and refinements of a setup similar to that described by Busch et al. (1999) and Kniep and Busch (1996). It resembles that of Emerson et al. (1994), designed to observe the responses of motile microbes to distinct gradients (Emerson et al., 1994), but it differs in that this apparatus immobilizes biological material as counterions meet across the immobilized biological material.

In this system, the gel functions to both (1) slow precipitation by retarding ion diffusion rates and (2) serve as a proxy for microbially produced polymeric substances, such as EPS (microbial extracellular polymeric substances), a matrix that is ubiquitous in microbial mats and biofilms. Considering the diffusion gel as the primary organic matrix, this setup will also accommodate secondary organics such as distinct EPS strands or pellets of microbial culture, which can be immobilized by slight heat fixation–adherence to the bottom cover slip of the assembly before addition of the diffusion gel. Staining of secondary organics may also be accommodated. The setup is small and easily handled and fits unobtrusively in a laboratory refrigerator for low-temperature experiments. However, extra care will need to be taken when imaging low-temperature experiments, as the gel may be heated by the light source. In this case, preliminary tests with the microscope are strongly advised to determine the degree to which this may occur and methods for ameliorating it (such as blowing cooled air between the light source and apparatus.) Nominal monitoring of gel temperature might be possible by measuring the temperature of the two cover slips by remote sensing, such as may be obtained by an infrared digital laser thermometer.

Experiment goals will dictate protocol details. For instance, after adhering a marine culture, gentle rinsing may be required to remove NaCl precipitates or growth media if their presence would interfere with the goal of the experiment.

A variety of polymeric substances are available for use, including lab standard polymers such as gelatin and agar, or custom organic substrates such as lab-grown EPS. The characteristics of each polymer are unique, most significantly in the nature and location of their charge balances – a discussion of which can be found in Kniep and Simon (2007) – and solubility (Whistler, 1973). The reader is referred to the book *Microbial Extracellular Polymeric Substances* (Wingender et al., 1999) for discussion of EPS and to Silverman and Boskey for a discussion of different polymers and the utility of DD setups in studying biomineralization. They also describe a constant-composition DD method comparing different proteins introduced into a gelatin matrix to illustrate their effects on calcium phosphate biomineralization (Silverman and Boskey, 2004).

## 3 Apparatus

### 3.1 Apparatus description

The active precipitation area of this setup is a thin diffusion gel, $\sim 1$ mm thick, sandwiched between a long coverslip and a square coverslip, into which ions are introduced from solution chambers via small channels leading into the gel (Fig. 1). Table 1 lists the required components. The setup block and adaptor–spacer can be easily made in-house. Detailed directions and solution concentrations suitable for reproducing our initial experiments can be found in the Supplement. The purchasable components of the setup are readily available.

### 3.2 Apparatus design considerations

The setup described here was designed with a cover slip bottom to allow use in an inverted microscope. Before constructing the setup, take into consideration the microscope that will be used and adjust dimensions as needed. For instance, an upright scope with objective lenses in a rotating turret may require a design with longer channels on either side of the center bore to avoid contact between the setup and the objective lenses.

If the precipitates are to be harvested from the diffusion gel for additional analysis, the solubility of the diffusion gel material should be taken into account. Gelatin is easily removed by repeated applications of hot water. Other gel materials are likely to require different treatments.

The success of this apparatus requires seals that are adequate for precluding leakage and evaporation and keeping the ion solutions from bypassing the diffusion gel and mixing prematurely. A great deal of trial and error went into this protocol, the results of which are incorporated into the Supplement step-by-step protocol. Please see the Supplement for details of the sealing method and precautions (Sect. S3.4 through S3.6) Assembly requires practice to achieve a full seal. Experimental details can be found in the Supplement.

## 4 Experimental results – an example

Our interest in developing this apparatus and protocol stemmed from our exploration of the influence of organics

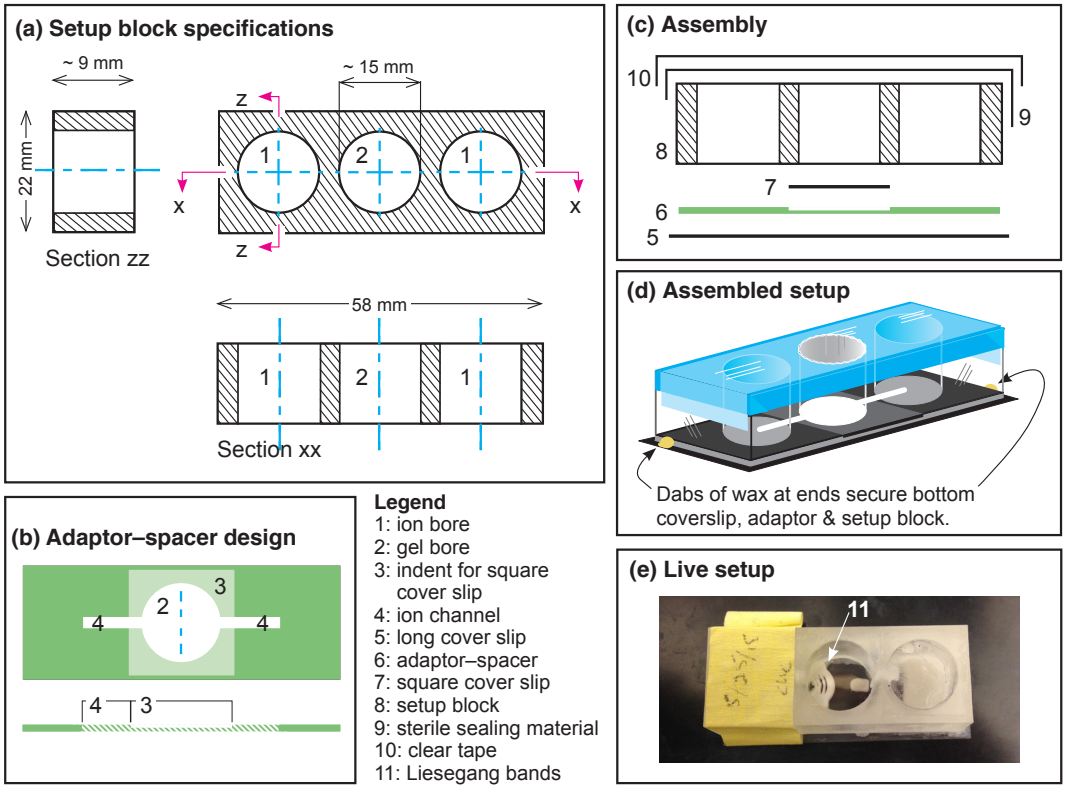


**Figure 1.** Specifications and assembly of unit. **(a)** Setup block diagram and specifications; **(b)** adaptor–spacer diagram; **(c)** exploded view showing assembly order; **(d)** assembled setup, isometric view; **(e)** a version of a live setup, viewed from above and showing Liesegang banding.

**Table 1.** Dimensions of the setup block and adaptor–spacer should be slightly narrower and shorter than the long coverslip.

| Setup components | | |
|---|---|---|
| Component | Material | Dimensions |
| Long coverslip | glass | 24 × 60 mm |
| Square coverslip | glass | 18 mm square |
| Setup block | plastic or glass | ∼ 22 × ∼ 58 mm (thickness: ∼ 3/8 in. or as preferred) |
| Adaptor–spacer | | silicone epoxy ∼ 22 × ∼ 58 mm (thickness: standard glass slide) |
| Assembly material | sterile bandaging* | > 24 × 60 mm |
| Assembly material | clear tape | 19 mm (sold as 3/4 in.) |
| Assembly material | Vaseline and lanolin | |

* Half of a 2–3/8 × 2–3/4 in. 3M Nexcare™ Tegaderm™ bandage, or similar, works well where sterility is desired, but its adhesive surface is designed to allow the escape of moisture. Clear watertight tape will seal it from evaporative loss. The bandage or tape plies can be punctured by needle for exchange of solutions during experimentation and then be resealed.

on the precipitation of calcium phosphates (apatite and its precursor phases.) We designed the apparatus to replicate conditions that might be present under primarily confining conditions such as within the microbial EPS of growing stromatolites or sediment pore spaces.

Early iterations of the apparatus with 9.5 mm (3/8 in.) gel depths formed a thick opaque cloud of precipitates, precluding optical microscopic imaging of precipitate details. In the assembly being described, the thickness of the gel is determined by the spacer used in forming the adaptor–spacer, as shown in Fig. S1a, in which a square cover slip is used to create the spacer inset into which the diffusion gel is added. This thinner gel thickness allows details of the development and maturation of the precipitation cloud that had been hidden in the deeper gels to be revealed. A similar, initially diffuse precipitation cloud forms, but then develops into a much more pronounced band with distinct boundaries before dividing into a number of discrete bands (Liesegang

bands), as shown in Fig. 1e. The phenomenon and dynamics of Liesegang banding remains an area of active research (Antal et al., 1999; Stern, 1954; Tripathi et al., 2015), and imaging and analysis of these separating Liesegang bands have shown differences in the size and morphology of the small constituent precipitates.

## 5 Conclusions

The features of this apparatus make it a versatile instrument for experiments in which microscopic observation of the precipitation process is desired. It is small and easily handled and fits unobtrusively in a laboratory refrigerator for low-temperature experiments. It requires only small amounts of diffusion gel and can accommodate secondary organics of interest. Experiments can be designed with any desired counterion solutions, the solution chemistry, pH, and Eh of which can be changed mid-experiment by needle and syringe. When utilized in conjunction with time-lapse microscopy, this apparatus provides an efficient and economical opportunity to observe and document mineral precipitation throughout the process.

*Data availability.* Because this manuscript describes the development of a new method for observing mineral precipitation, links to data are not applicable here. All specifications required to reproduce the apparatus are included in the Supplement. If readers have questions about the procedures, or any of the images included here, they are welcome to contact the corresponding author.

**The Supplement related to this article is available online at doi:10.5194/bg-14-2151-2017-supplement.**

*Author contributions.* Jake V. Bailey contributed the initial experimental goal. Chris H. Crosby developed the apparatus and protocol. Chris H. Crosby and Jake V. Bailey wrote the paper.

*Competing interests.* The authors declare that they have no conflict of interest.

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## References

Antal, T., Droz, M., Magnin, J., and Rácz, Z.: Formation of Liesegang patterns: A spinodal decomposition scenario, Phys. Rev. Lett., 83, 2880–2883, doi:10.1103/PhysRevLett.83.2880, 1999.

Becker, A., Becker, W., Marxen, J. C., and Epple, M.: In-vitro crystallization of calcium carbonate in the presence of biological additives – Comparison of the ammonium carbonate method with double-diffusion techniques, J. Inorg. Gen. Chem., 629, 2305–2311, doi:10.1002/zaac.200300229, 2003.

Busch, S., Dolhaine, H., Duchesne, A., Heinz, S., Hochrein, O., Laeri, F., Podebrad, O., Vietze, U., Weiland, T., and Kniep, R.: Biomimetic morphogenesis of fluorapatite-gelatin composites: Fractal growth, the question of intrinsic electric fields, core/shell assemblies, hollow spheres and reorganization of denatured collagen, Eur. J. Inorg. Chem., 1999, 1643–1653, doi:10.1002/(SICI)1099-0682(199910)1999:10<1643::AID-EJIC1643>3.0.CO;2-J, 1999.

Emerson, D., Worden, R. M., and Breznak, J. A.: A diffusion gradient chamber for studying microbial behavior and separating microorganisms, Appl. Environ. Microbiol., 60, 1269–1278, 1994.

Hunter, G. K., Allen, B. L., Grynpas, M. D., and Cheng, P.-T.: Inhibition of hydroxyapatite formation in collagen gels by chondroitin sulphate, Biochem. J., 228, 463–469, 1985.

Kniep, R. and Busch, S.: Biomimetic growth and self-assembly of fluorapatite aggregates by diffusion into denatured collagen matrices, Angew. Chemie Int. Edit., 108, 2623–2626, 1996.

Kniep, R. and Simon, P.: Fluorapatite-gelatine-nanocomposites: Self-organized morphogenesis, real structure and relations to natural hard materials, Top. Curr. Chem., 270, 73–125, doi:10.1007/128, 2007.

Morse, J. W.: Dissolution kinetics of calcium carbonate in sea water: III, a new method for the study of carbonate reaction kinetics, Am. J. Sci., 274, 97–107, 1974.

Silverman, L. and Boskey, A. L.: Diffusion systems for evaluation of biomineralization, Calcified. Tissue Int., 75, 494–501, doi:10.1007/s00223-004-0019-y, 2004.

Stern, K. H.: The Liesegang phenomenon, Chem. Rev., 54, 79–99, 1954.

Tomson, M. B. and Nancollas, G. H.: Mineralization Kinetics: A constant composition approach, Science, 200, 1059–1060, 1978.

Tripathi, A., Srivastava, G., Srivastava, S., and Das, I.: Liesegang patterns, growth kinetics, inhibition and dissolution of calcium phosphate: A constituent of renal stone, Chem. Biol. Lett., 2, 30–40, 2015.

Whistler, R. L.: Solubility of polysaccharides and their behavior in solution, in: Carbohydrates in Solution, edited by: Isbell, H. S., American Chemical Society, Washington, DC, 242–255, 1973.

Wingender, J., Neu, T. R., and Flemming, H.-C. (Eds.): Microbial Extracellular Polymeric Substances – Characterization, Structure and Function, Springer-Verlag, Berlin, 1999.